PCR of pIKM1

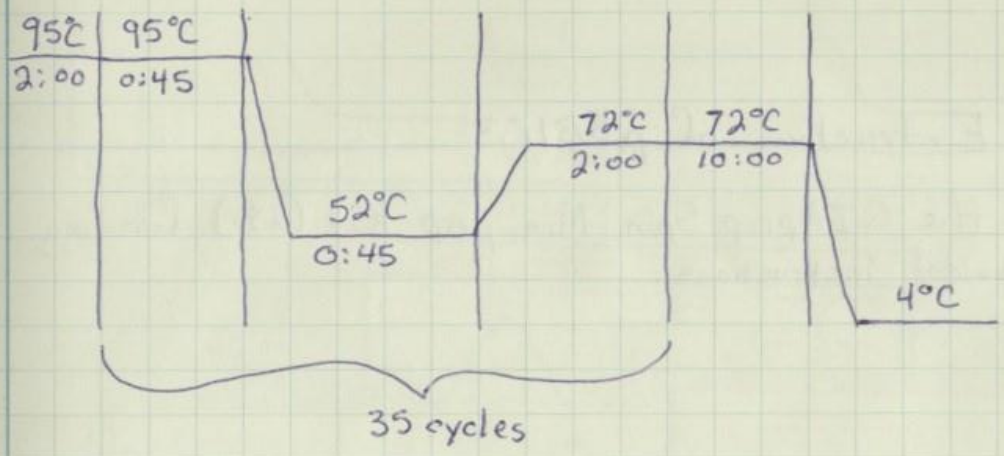
2/19/14

150 µL of 2x Master Mix
1 µL of Forward Primer
1 µL of Reverse Primer
148 µL of PCR water
300 µL Total PCR Mix

Primers:

- Clos_ORI_F_BioBrick
- BioBrick_OriClos_R_V3

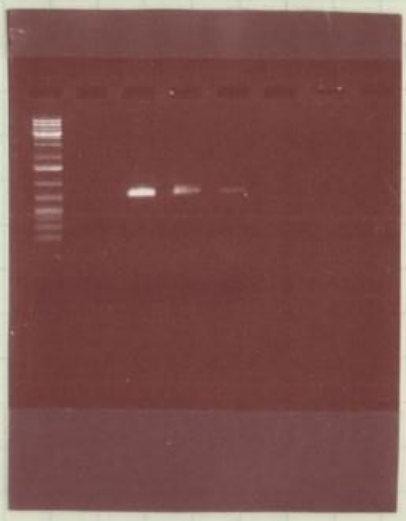
- 4 PCR tubes total. 1 tube should be a control.
- Make dilutions of template 1:10, 1:100, + 1:1000.
- Add 50 µL of PCR Mix to each tube
- Add 1 µL of template dilution to it's own PCR tube respectively. Do not add to control.



Gel of pIKM1

2/20/14

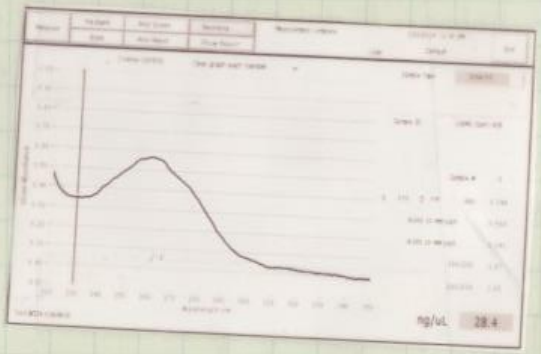
Row 1
Lane 1 – 1kb Plus
Lane 2 – Control
Lane 3 – 1:10 pIKM1
Lane 4 – 1:100 pIKM1
Lane 5 – 1:1000 pIKM1
Lane 6 – Empty
Lane 7 – Empty
Lane 8 – Empty



2/20/14 — PCR Cleanup of pIKM1

Used the QIAquick PCR Purification Kit (250) following the provided instructions.

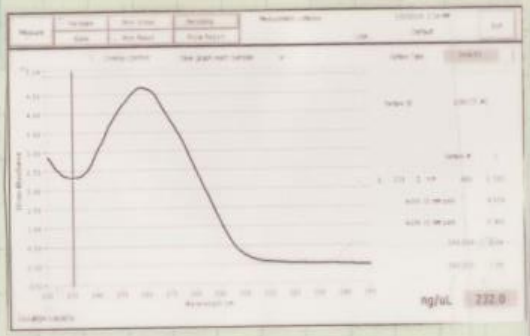
2/20/14 — Quantification of pIKM1

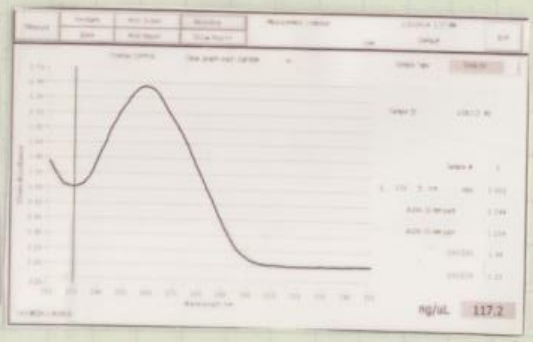


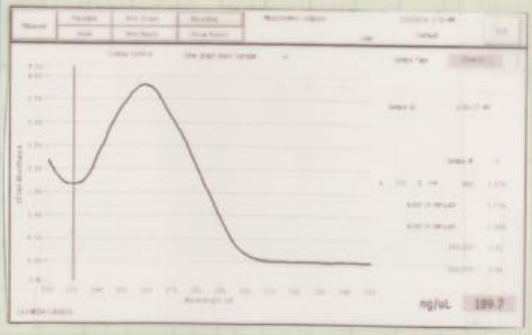
No good.
Repeat PCR with only 1:1.

2/20/14 — DNA Extraction of pSB1C3

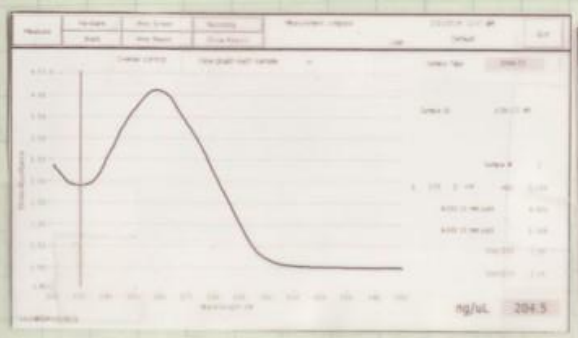
Used the QIAprep Spin Miniprep Kit (250) following the provided instructions.

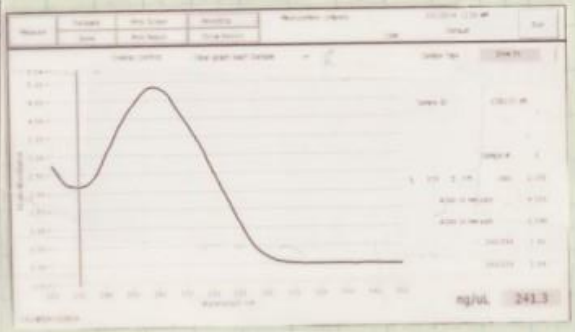


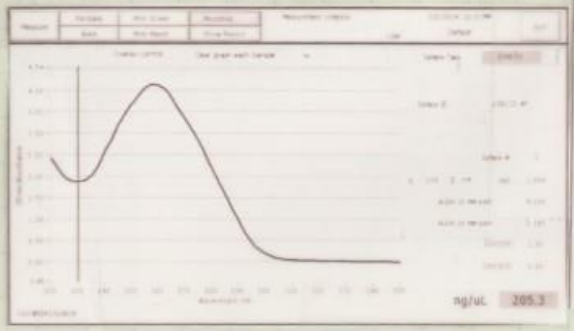


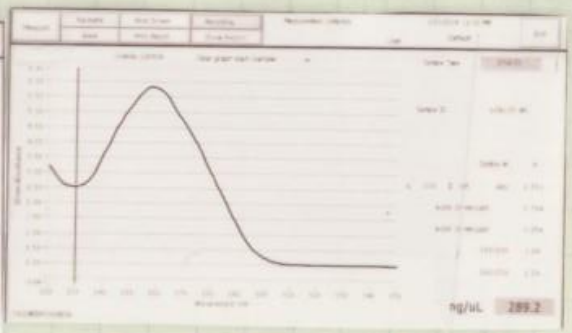


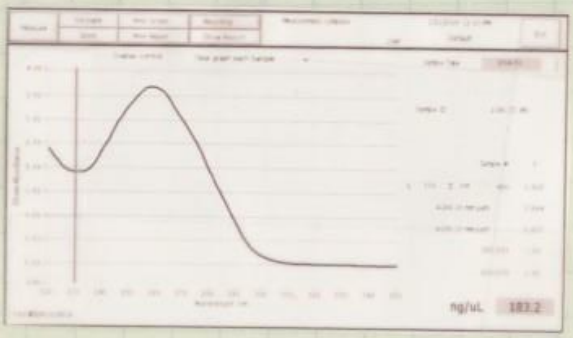
2/20/14











2/20/14

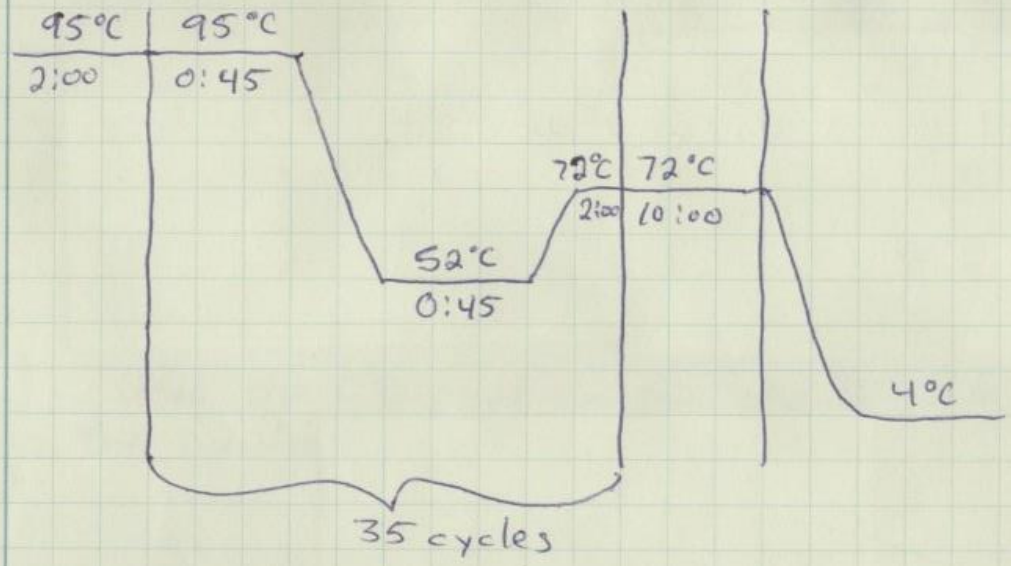
2nd PCR of pIKM1 (clos ori)

150 µL of 2x Master Mix
1 µL of Forward Primer
1 µL of Reverse Primer
148 µL of PCR Water
300 µL Total PCR mix

primers:

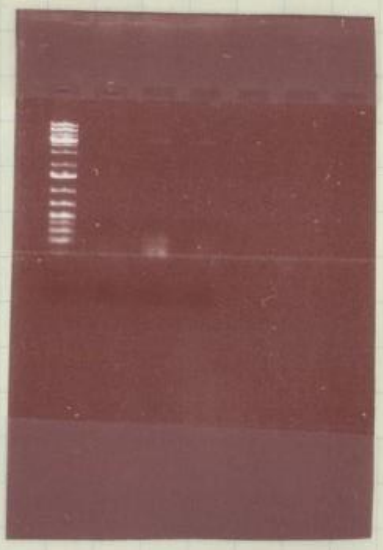
- Clos_ORI_F_BioBrick
- BioBrick_OriClos_R_V3

Use 1:1 of Template pIKM1 (twice with a control.)



Gel of pIKM1 (Clos Ori)

Row 1
Lane 1 - 1kb Plus
Lane 2 - Control
Lane 3 - Clos Ori (A)
Lane 4 - Clos Ori (B)
Lane 5 - Empty
Lane 6 - Empty
Lane 7 - Empty
Lane 8 - Empty



Why is this PCR worse?!

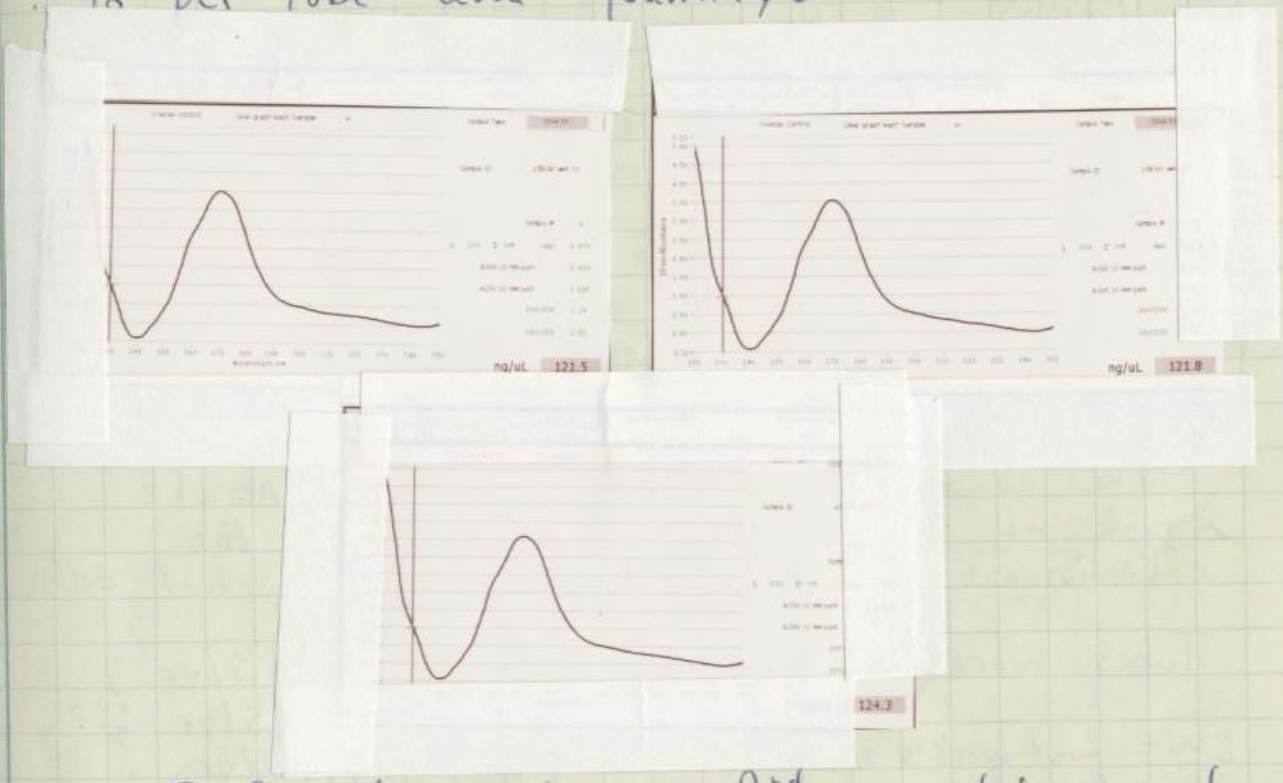
Rescuing pSB1A3, pSB1K3, pSB1T3 from the Registry

2/24/14

From the Spring 2013 stocks sent to us from the iGEM registry, pSB1A3, pSB1K3 and pSB1T3 were rescued from the 5th plate.

pSB1A3	Location :	Plate 5	well 1G
pSB1K3	Location :	Plate 5	well 5A
pSB1T3	Location :	Plate 5	well 7A

Penetrate metal foil with pippette tip (steeile) filled with 10μL of Crosslinked PCR water. Add water to well and pippette up and down a few times to mix dry DNA and let sit in the well before extracting. Add the 10μL from the well to PCR tube and quantify.



Tranfer 6μL to a 2nd pcr tube and use the equations

$$V_1C_1 = V_2C_2 \quad \text{or} \quad \frac{6\,\mu L\,(x\ ng/\mu L)}{100\ ng/\mu L}$$

to fix the concentration to 100ng/μL.

2/24/14

Transforming pSB1A3, pSB1K3 + pSB1T3

- pSB1A3 at 121.5 ng/μL = C_1, 6μL = V_1 and 100ng/μL = C_2. $V_2 = x$

$\frac{6(121.5)}{100} = 7.29\mu L - 6\mu L =$ add 1.29μL PCR H_2O

- pSB1K3 at 121.8ng/μL = C_1, 6μL = V_1 and 100ng/μL = C_2. $V_2 = x$

$\frac{6(121.8)}{100} = 7.30\mu L - 6\mu L =$ add 1.3μL PCR H_2O

- pSB1T3 at 124.3ng/μL = C_1, 6μL = V_1, and 100ng/μL = C_2. $V_2 = x$

$\frac{6(124.3)}{100} = 7.46\mu L - 6\mu L =$ add 1.46μL PCR H_2O

1μL each of pSB1A3 goes into the competent cells made in 2013 of 100μL, 1μL of pSB1A3 goes into 100μL of competent cells made 2/14/14, and 1μL goes into 100μL of competent cells made 2/19/14 for transformation. Repeat process for pSB1K3 + pSB1T3. There was a total of 9 ~~repeat~~ seperate transformations.

The reasoning for using 3 ~~sepea~~ seperate stocks of Top10 cells was to compare ~~the~~ effectiveness of the new batches of competent cells.

Tubes were labelled: A3 LW, A3 2W, A3 1yr;
K3 LW, K3 2W, K3 1yr;
T3 LW, T3 2W, T3 1yr.

LW = Last week's stock
2W = 2 weeks ago stock
1yr = last years stock

referring to Top 10 batches.

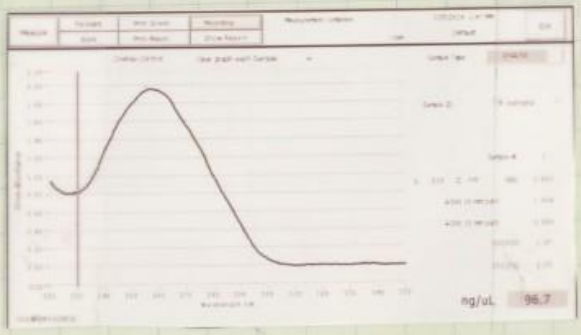
- After the addition of template to Top10 cells incubate on ice for 30 min.
- Heat shock in water bath at 37°C for 60 seconds.
- Immediately transfer the tubes back on ice (in ice bucket) for 5 minutes.
- Add 600µL of psi broth and then incubate at 37°C for 2 hours at 200 rotations per minute.
- After incubation make 1:1 and 1:10 dilutions and plate them with respect to the correct antibiotic plate.

  * There should be a total of 18 plates.

DNA extraction of R. eutropha

2/25/14

Used the QIA prep Spin Mini prep Kit (250) following the provided instructions.



2/26/14

Transforming pSB1A3 + pSB1K3 continued

- The transformation for pSB1T3 appeared successful as indicated by the pink growth caused by the RFP.
- However It is highly suspected that the K3 backbones were grown on Ampicillin plates and A3 backbones were grown on Kanamycin plates. This will obviously result in no growth.
- Let it be noted that the transformations for these backbones were saved in the -20°C freezer. They are to be diluted again and restreaked.

* Also the results of the pSB1T3 transformation gave a few colonies, but I feel that the concentration of template used in future gene rescues should be much greater. (or at least the nanograms should be greater).

- Restreak pSB1A3 + pSB1K3 onto their respective antibiotic plates.

- Results: pink growth. Victory!

BktB PCR (whole gene template)

2/26/14

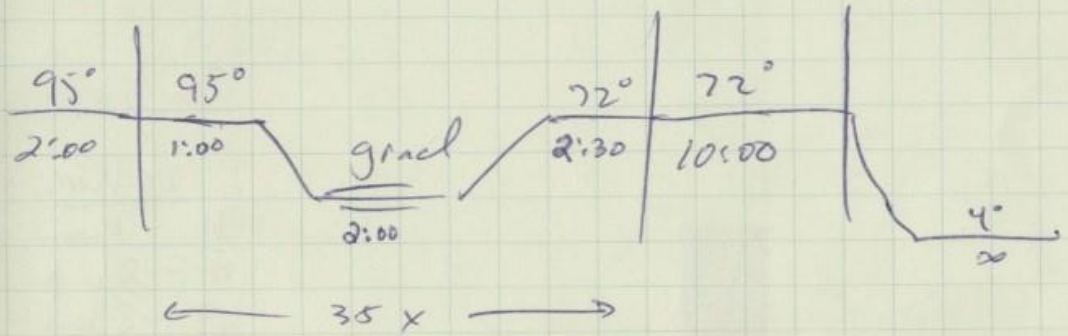
Needed to amplify BktB to use for future template and sequencing

Mix:

480 µL	2x mm
5 µL	BktB FWD primer
5 µL	BktB REV primer
460 µL	dH_2O
10 µL	Template
960 µL	

PCR on gradient 50° → 60°

8 Rxns, positions 1, 3, 5, 6, 7, 8, 10, 12



Jeremiah's PCR of BktB. → This will be one of my new projects. Next steps will be to run a gel, pCR clean-up, and a digestion with PstI. Using another Gel we can tell if the BktB gene has 2 or 3 restriction sites.

2/26/14

Gel of BktB PCR

The Numbers refer to the positions in the thermocycler on a gradient, 1 on the far left and 12 on the far right.

Row 1

Lane 1 – 1kb Plus
Lane 2 – #1
Lane 3 – #3
Lane 4 – #5
Lane 5 – #6
Lane 6 – Empty
Lane 7 – Empty
Lane 8 – Empty

Row 2

Lane 1 – 1kb Plus
Lane 2 – #7
Lane 3 – #8
Lane 4 – #10
Lane 5 – #12
Lane 6 – Empty
Lane 7 – Empty
Lane 8 – Empty

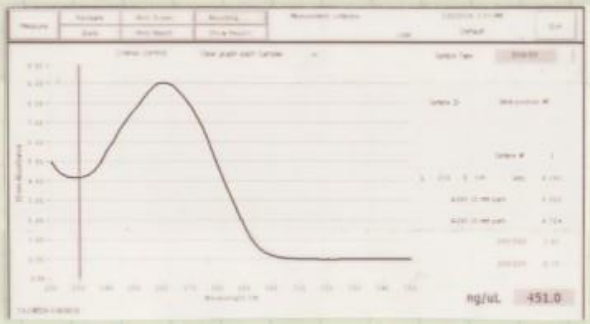
2/26/14

Digest of BktB

Digest only #3

PstI	FB 10x	DNA	PCR H_2O
2μL	2μL	1.11μL	14.89μL

Total of 20μL



$500\,ng \left| \frac{1\,uL}{451\,ng} \right. = 1.11\,uL$

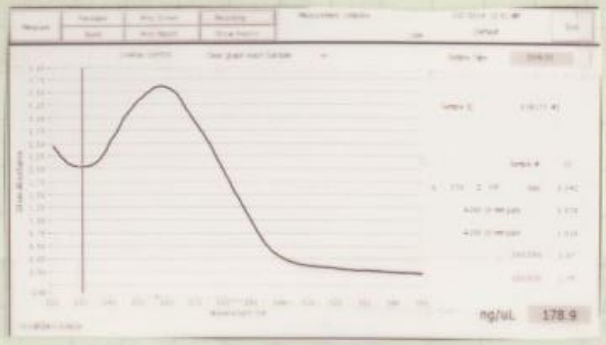
- Digest in heat bath @ 37°C for 50min.
- The heat kill the enzyme on the heat block at 80°C for 15 min.

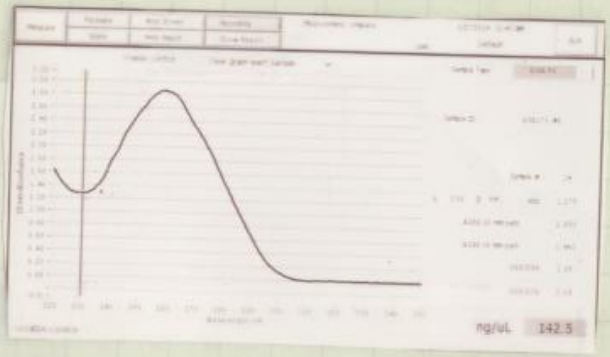
Gel of BktB Digest — 2/26/14

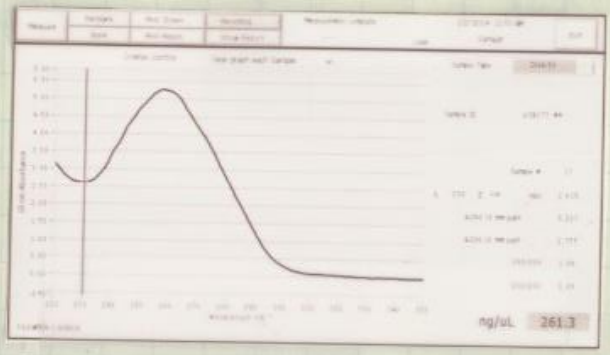
Row #1
Lane 1 – 1kb Plus
Lane 2 – #3
Lane 3 – Empty
Lane 4 – Empty
Lane 5 – Empty
Lane 6 – Empty
Lane 7 – Empty
Lane 8 – Empty

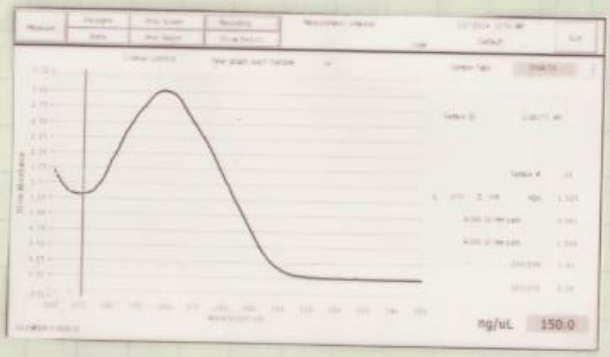
BktB gene was probably too diluted to show on gel. Try again later with a more concentrated dilution.

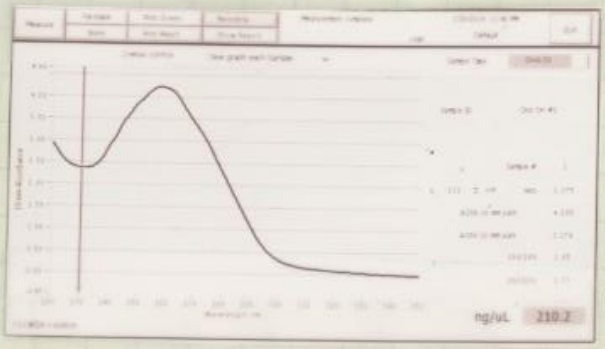
2/26/14 DNA Extraction of pSB1T3 and Clos Ori

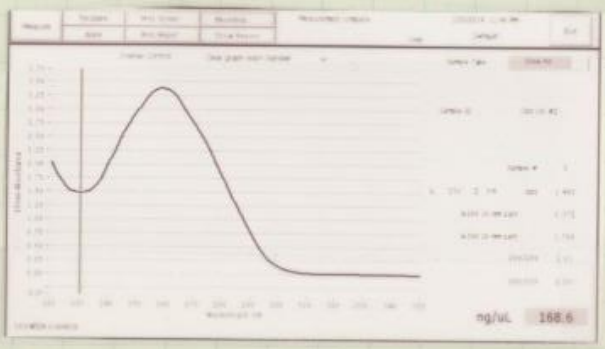


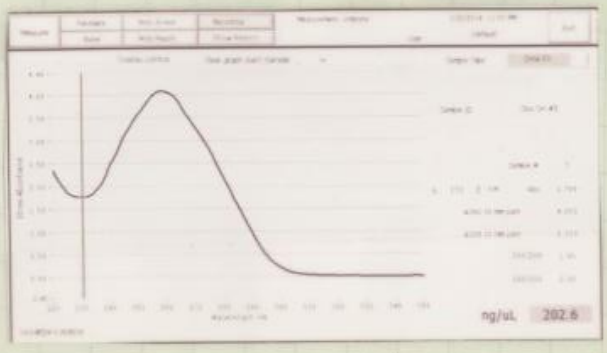




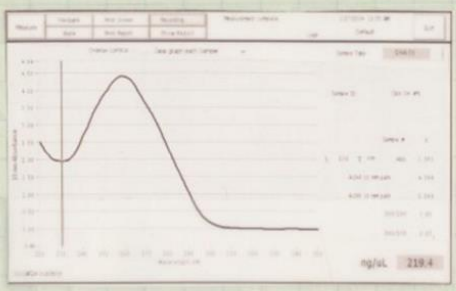




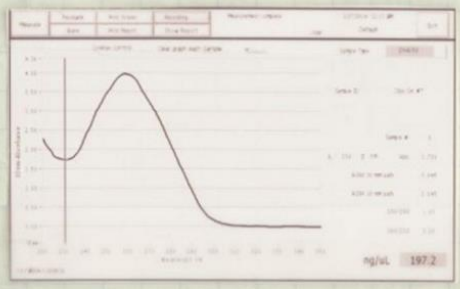


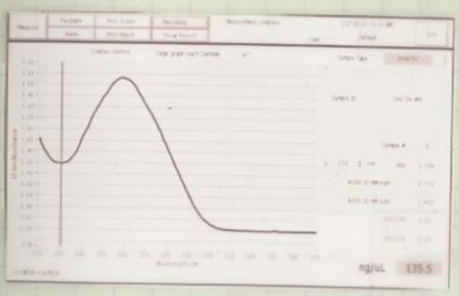


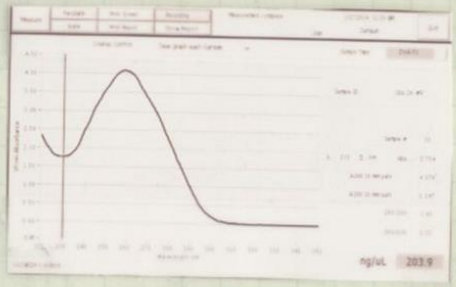

ng/uL 80.0


ng/uL 219.4


ng/uL 241.8


ng/uL 197.2


ng/uL 135.5


ng/uL 203.9


ng/uL 143.3


ng/uL 291.0

2/27/14

Digest of Clos Ori

Clos Ori #1 $500\,ng \left| \frac{1\,\mu L}{210.2\,ng} \right. = 2.38\,\mu L$

Clos Ori #2 $500\,ng \left| \frac{1\,\mu L}{168.6\,ng} \right. = 2.97\,\mu L$

Clos Ori #3 $500\,ng \left| \frac{1\,\mu L}{202.6\,ng} \right. = 2.47\,\mu L$

Clos Ori #4 $500\,ng \left| \frac{1\,\mu L}{80.0\,ng} \right. = 6.25\,\mu L$

Clos Ori #5 $500\,ng \left| \frac{1\,\mu L}{219.4\,ng} \right. = 2.28\,\mu L$

Clos Ori #6 $500\,ng \left| \frac{1\,\mu L}{241.8\,ng} \right. = 2.07\,\mu L$

Clos Ori #7 $500\,ng \left| \frac{1\,\mu L}{197.2\,ng} \right. = 2.54\,\mu L$

Clos Ori #8 $500\,ng \left| \frac{1\,\mu L}{135.5\,ng} \right. = 3.69\,\mu L$

Clos Ori #9 $500\,ng \left| \frac{1\,\mu L}{203.9\,ng} \right. = 2.45\,\mu L$

~~Cto~~

Clos Ori #10 $500\,ng \left| \frac{1\,\mu L}{143.3\,ng} \right. = 3.49\,\mu L$

Clos Ori #11 $500\,ng \left| \frac{1\,\mu L}{291.0\,ng} \right. = 1.72\,\mu L$

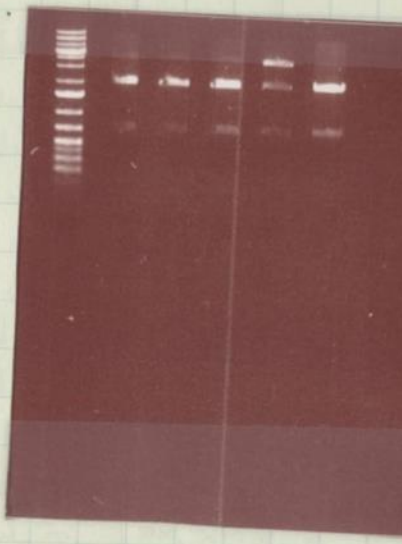
sample	~~Pst I~~ Spe I	EcoRI	10x buffer	DNA	PCR H_2O	Total
ClosOri #1	2 µL	2 µL	2 µL	2.38 µL	11.62	20 µL
ClosOri #2	2 µL	2 µL	2 µL	2.97 µL	11.03	20 µL
ClosOri #3	2 µL	2 µL	2 µL	2.47 µL	11.53	20 µL
ClosOri #4	2 µL	2 µL	2 µL	6.25 µL	7.75	20 µL
ClosOri #5	2 µL	2 µL	2 µL	2.28 µL	11.72	20 µL
ClosOri #6	2 µL	2 µL	2 µL	2.07 µL	11.93	20 µL
ClosOri #7	2 µL	2 µL	2 µL	2.54 µL	11.46	20 µL
ClosOri #8	2 µL	2 µL	2 µL	3.69 µL	10.31	20 µL
ClosOri #9	2 µL	2 µL	2 µL	2.45 µL	11.55	20 µL
ClosOri #10	2 µL	2 µL	2 µL	3.49 µL	10.51	20 µL
ClosOri # 11	2 µL	2 µL	2 µL	1.72 µL	12.28	20 µL

- Digest in heat bath at 37°C for 50 minutes.
- Heat kill enzymes on heat block at 80°C for 15 minutes.

~~Gel of~~ ClosOri 2/2

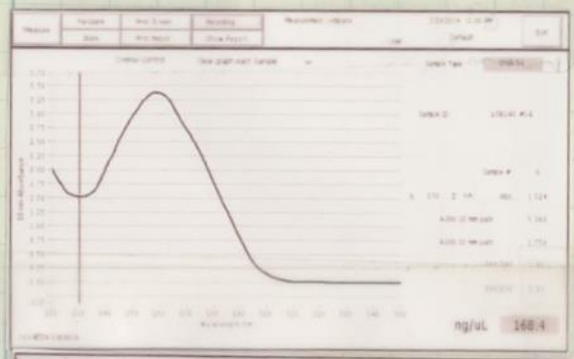
Row 1
Lane 1 - 1kbPlus
Lane 2 - clos ori #1
Lane 3 - clos ori #2
Lane 4 - clos ori #3
Lane 5 - clos ori #4
Lane 6 - clos ori #5
Lane 7 - clos ori #6
Lane 8 - Empty

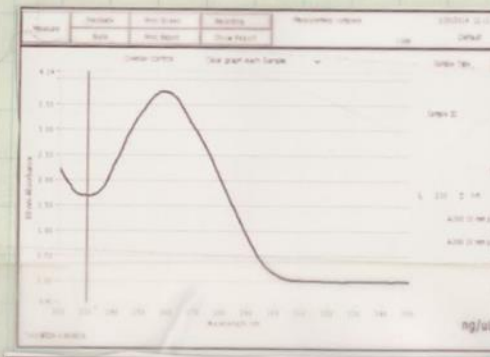
Row 2
Lane 1 - 1Kb Plus
Lane 2 - closori #7
Lane 3 - closori #8
Lane 4 - closori #9
Lane 5 - closori #10
Lane 6 - closori #11
Lane 7 - Empty
Lane 8 - Empty

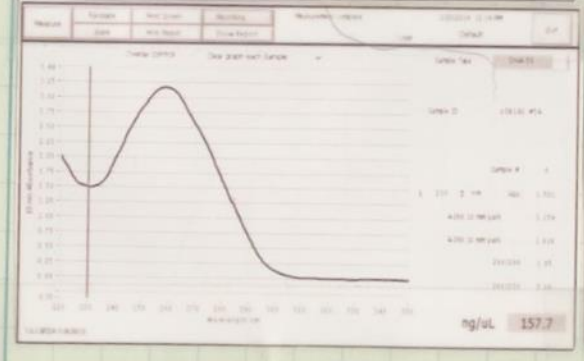


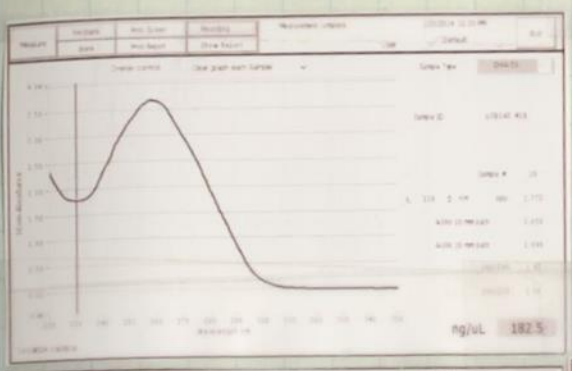
2/27/14 DNA extraction of pSB1A3

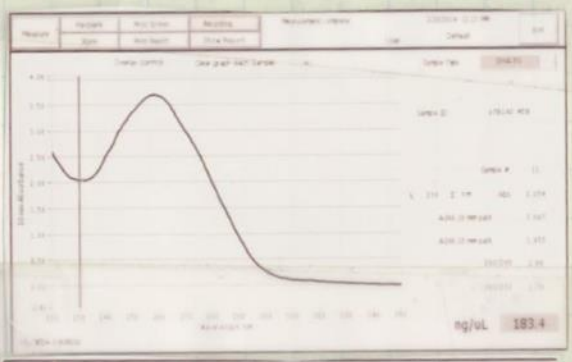
Used the QIA prep Spin Miniprep K
(250) folling the provided instructions.

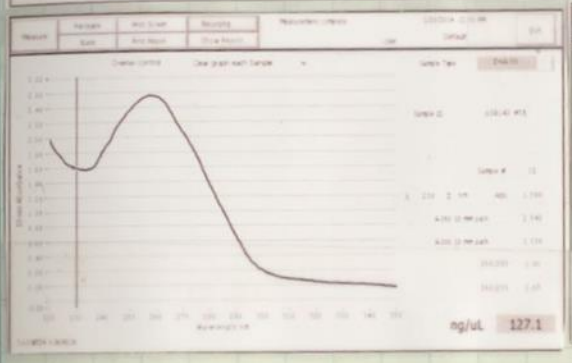


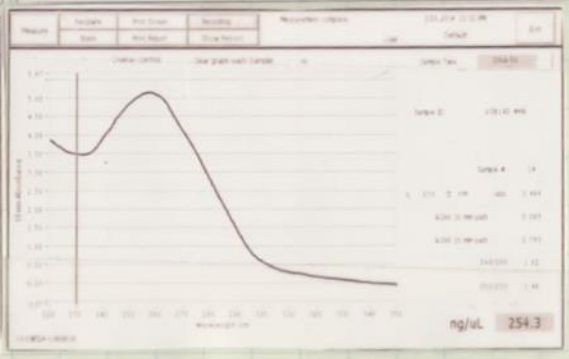










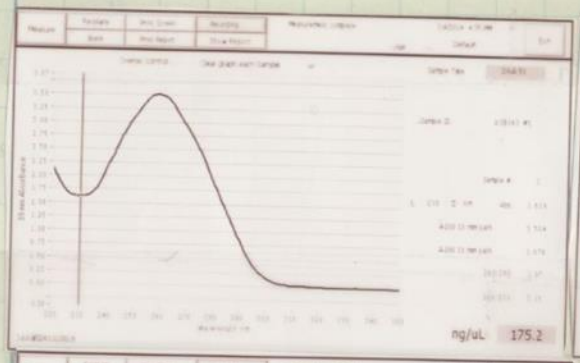


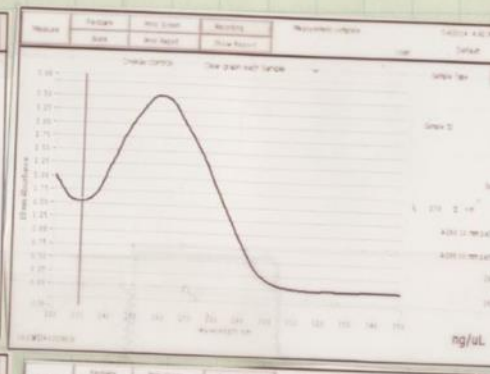
At this point we ~~ten~~ now have all of the promoters in pSB1C3, all of the terminators in pSB1C3, mlsR in pSB1C3, Clos Ori in pSB1C3, and the four antibiotic resistant backbones pSB1A3, pSB1K3, pSB1C3, and pSB1T3.

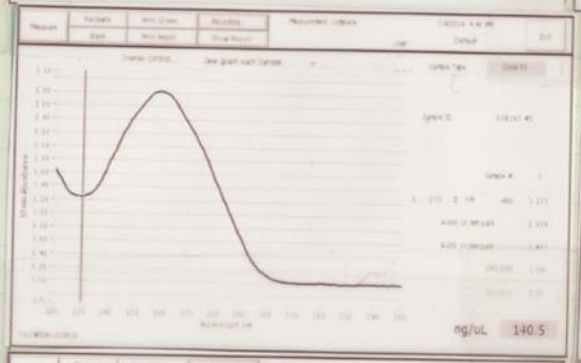
2/29/14

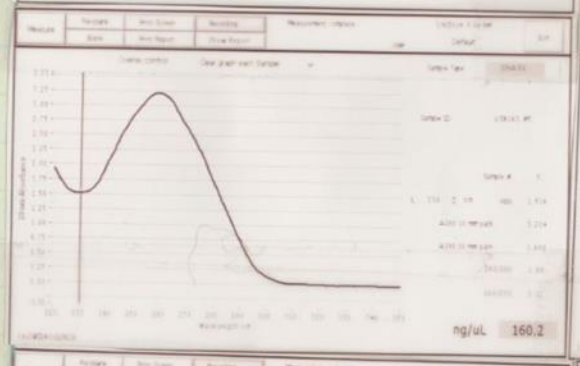
DNA Extraction of pSB1K3

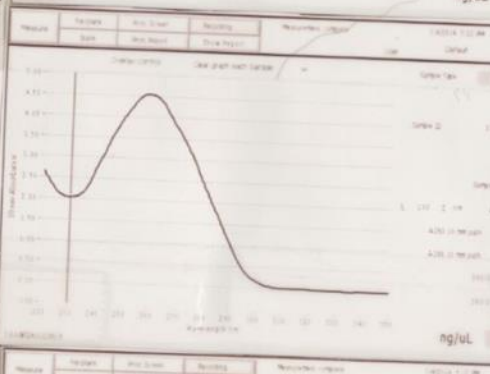
The QIA prep Spin Miniprep Kit (
following the provided instructions.

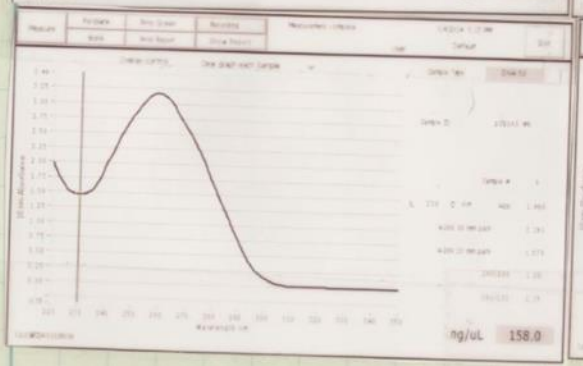


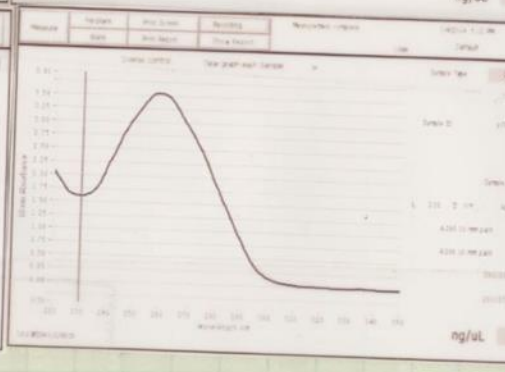


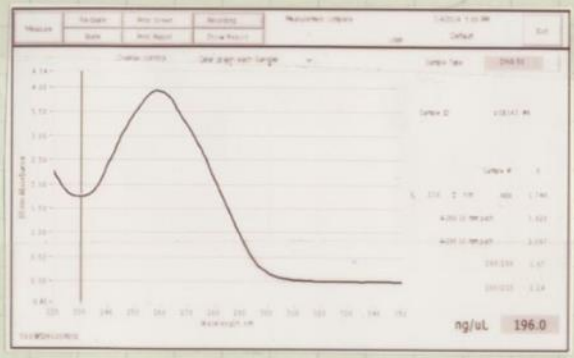












Future Steps for Plasmid Construction 3/1

Previously it was believed that the upstream part and the down stream part had to be in seperate plasmids prior to 3A assembly. We found recently that this was not true. Therefore 3A assembly will begin as soon as we get more T4 Ligase and Pst I restriction enzyme that can be heat killed.

Digest of Clos ori and Ptet- for 3A 3/27

500 ng	Upstream part (Clos ori)
1 µL	Eco RI
1 µL	~~Pst I~~ Spe I
5 µL	10 x Buffer
1 µL	BSA
add to 50µL	PCR H_2O

500 ng	Downstream part (Ptet-)
1 µL	~~X~~ba I
1 µL	Pst I
5 µL	10x Buffer
1 µL	BSA
add to 50µL	PCR H_2O

50 ng	Backbone Plasmid

Clos Ori $500\,ng \left| \frac{1\,\mu L}{210.2\,ng} \right. = 2.38\,\mu L$ in 3 of H_2O

P_{ptb-} #9 $500\,ng \left| \frac{1\,\mu L}{235.7\,ng} \right. = 2.12\,\mu L$ in 3 of H_2O

pSB1T3#1 $500\,ng \left| \frac{1\,\mu L}{178.9\,ng} \right. = 2.79\,\mu L$ in 3 of H_2O

- Place in heat bath for 45min at 42°C.
- Heat kill enzymes on heat block for 15min 80°C.

3/27/14 Ligations of 3A assembly

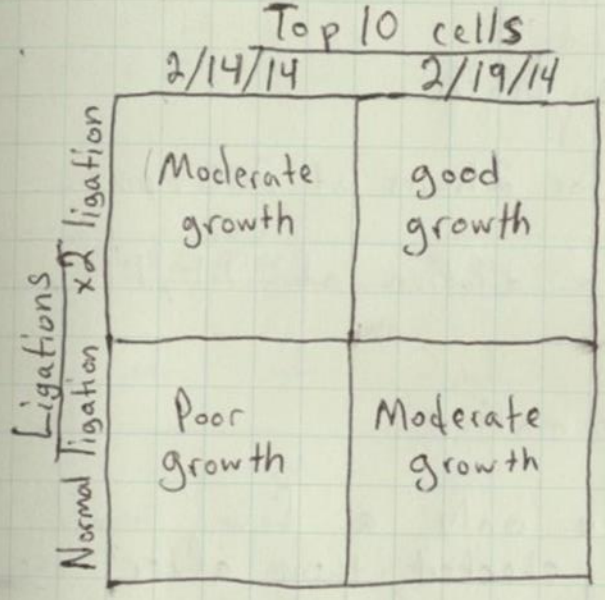
The protocol I found for 3A ligation c for the following amounts.

2μL of upstream part
2μL of downstream part
2μL of destination backbone
2μL of 10x T4 ligase buffer
0.5μL of T4 ligase
11μL of H_2O

The 0.5μL increment seemed small enough t allow for a great chance in pipetting errors.
∴ I conducted two ligations. one ligation using the above protocol, and the second us a double sized reaction Ligation. Each ligati will be used to compare the transformation efficiency between the two

4μL of upstream part
4μL of downstream part
4μL of destination backbone
4μL of 10x T4 ligase buffer

1μL of T4 ligase
22μL of H_2O



Future Transformation Matrix

- Incubate on benchtop for 20 min.
- Ready to transform.

Transformation of 3A assembly

Since this is a very inefficient procedure, a total of 10 transformations were conducted all at once. These trasformations are ~~the~~ product of the matrix created in the Ligation step.

1 Normal Ligation = NL + 2/14 #1
1 Normal Ligation = NL + 2/14 #2

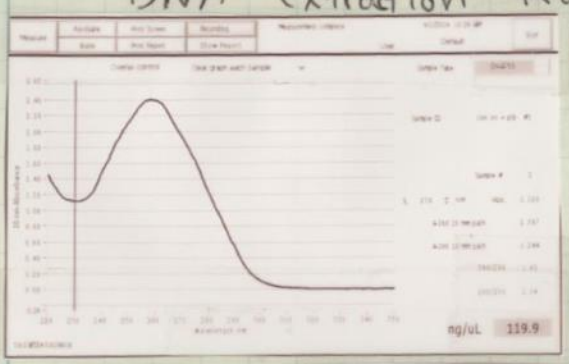
- Trasform 4 μL of Ligation product into of competent cells for each transformation
- Keep on ice for 30 min.
- Heat shock in water bath for 60 sec a
- Place back on ice for 5 min.
- add 600 μL of psi broth.
- Incubate at 37°C for 2 hours at 200 r
- Then plate using a dilution of 1:1,

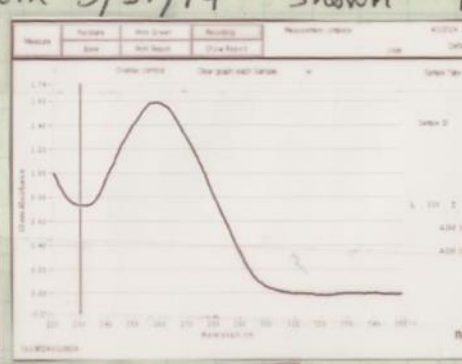
3/31/14 Results of Transformation

At first there were only a few s colonies, but when I checked them afte weekend it appears the transformations w very efficient because the plates were in colonies.

4/1/14 Restriction Digest of 3A assembled parts

DNA extraction from 3/31/14 shown





Clos Ori + Ptb- #1 $500\,ng \left| \frac{1\,\mu L}{119.9\,ng} \right. = 4.17\,\mu L$

Clos Ori + Ptb- #2 $500\,ng \left| \frac{1\,\mu L}{78.9\,ng} \right. = 6.34\,\mu L$

Clos Ori + Ptb- #3 $500\,ng \left| \frac{1\,\mu L}{112\,ng} \right. = 4.46\,\mu L$

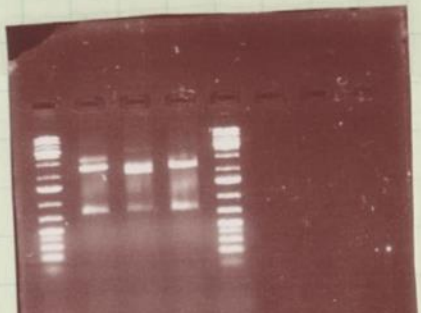
Sample	EcoRI	SpeI	10x buffer	BSA	PCR H_2O	DNA
#1	1 µL	1 µL	2 µL	1 µL	10.83 µL	4.17 µL
#2	1 µL	1 µL	2 µL	1 µL	8.66 µL	6.34 µL
#3	1 µL	1 µL	2 µL	1 µL	10.54 µL	4.46 µL

- Place in heatbath for 45 min at 42°C.
- Heat kill enzymes on heat block for 15 min at 80°C

4/1

Gel of Clos Ori + Ptb-

Row 1
Lane 1 - 1 kb Plus
Lane 2 - #1
Lane 3 - #2
Lane 4 - #3
Lane 5 - 1 kb Plus



4/11/14 — Sending 3A for Sequencing

Sent Samples #1, and #3 for sequenc
Sample #2 has a low concentration and
be sent if #1 - #3 are not confirmed.

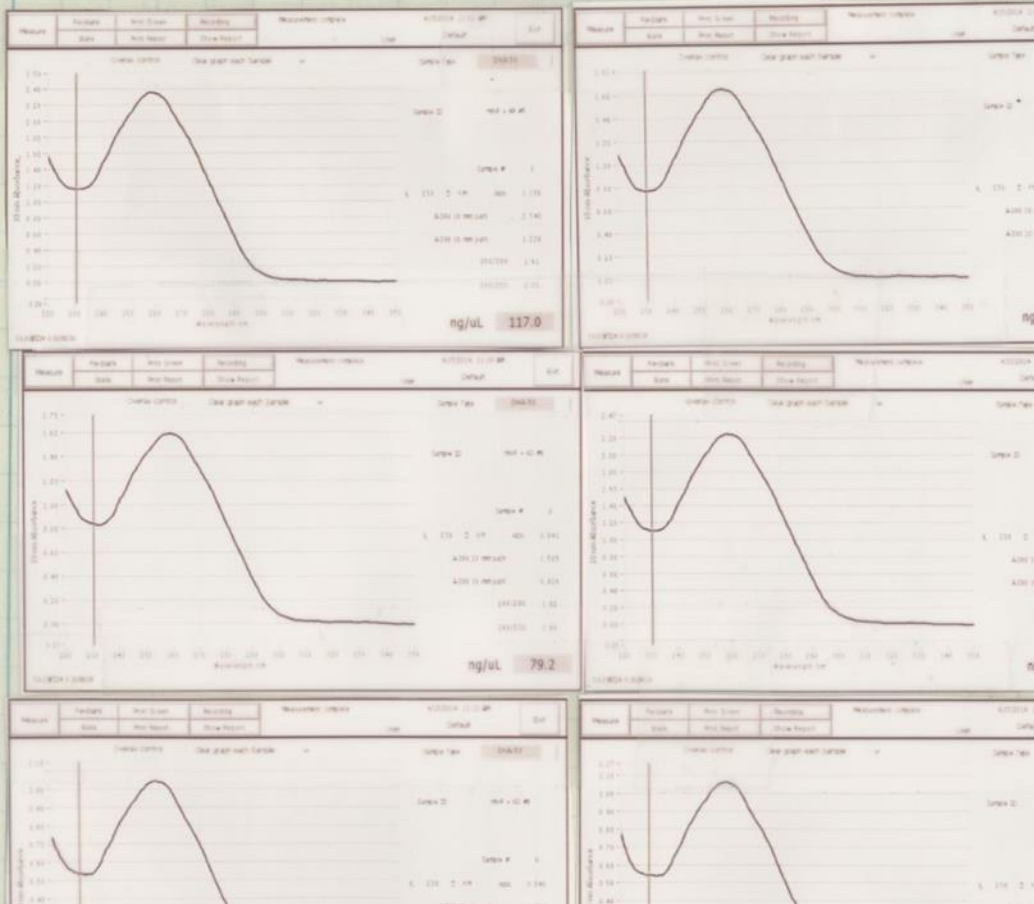
Results of Sequencing

#1 - Unknown 4/15/14

#3 - Confirmed !! 4/15/14

VICTORY!!!

4/15/14 DNA Extraction [redacted] of 3A mlsR +4P. and mlsR 6D



Digest of 3A mlsR+4P and mlsR+6D

4/16

mlsR + 4P #3 $500 \left| \frac{1\ \mu L}{117\ ng} \right. = 4.27\ \mu L$

mlsR +4P #4 $500 \left| \frac{1\ \mu L}{112\ ng} \right. = 4.46\ \mu L$

mlsR +4P #7 $500 \left| \frac{1\ \mu L}{82\ ng} \right. = 6.10\ \mu L$

mlsR +6D #9 $500 \left| \frac{1\ \mu L}{79.2\ ng} \right. = 6.31\ \mu L$

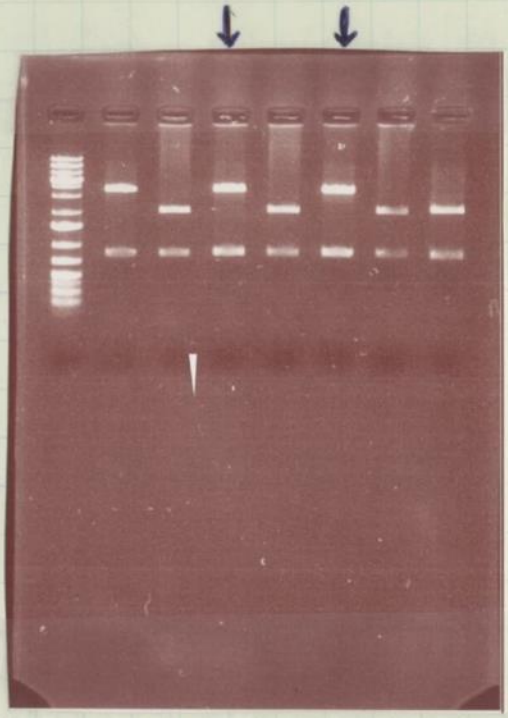
mlsR #24 $500 \left| \frac{1\ \mu L}{455.1\ ng} \right. = 1.10\ \mu L$

Sample	EcoRI	PstI	10x buffer	BSA	PCR H_2O	DNA
- mlsR+4P#3	1 μL	1 μL	2 μL	1 μL	10.73 μL	4.27 μL
- mlsR+4P#4	1 μL	1 μL	2 μL	1 μL	10.54 μL	4.46 μL
- mlsR+4P#7	1 μL	1 μL	2 μL	1 μL	8.9 μL	6.10 μL
- mlsR+6D#9	1 μL	1 μL	2 μL	1 μL	8.69	6.31 μL
- mlsR #24	1 μL	1 μL	2 μL	1 μL	13.9 μL	1.10 μL

- Digest in water bath for 42°C for 45 min
- Heat block @ 80°C for 15 min

4/16/14 Gel of 3A mlsR+4P and mlsR+6D

Row 1



Lane 1 - 1kb Plus
Lane 2 - mlsR+4P#3
Lane 3 - mlsR [redacted] #24
Lane 4 - mlsR+4P#4
Lane 5 - mlsR #24
Lane 6 - mlsR+4P#7
Lane 7 - mlsR #24
Lane 8 - mlsR+6D#9

Conclusion: Lanes 4 and 6 have inserts tha
are close to out target piece.

* send mlsR+4P#4 and mlsR+4P#7

4/18/14 3A of mlsR+4P Sequencing

Sent Samples mlsR+4P#4 and mlsR+4P
for sequencing

Results of sequencing

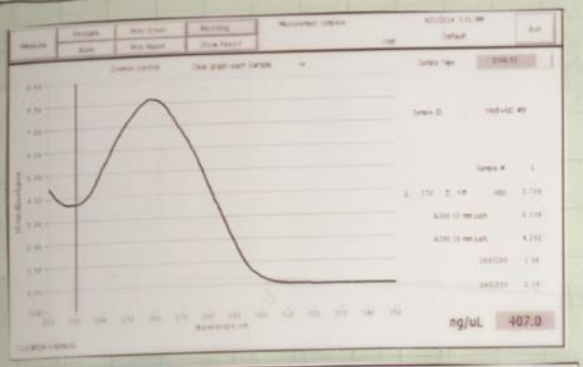
#4 - Negative 4/18/14

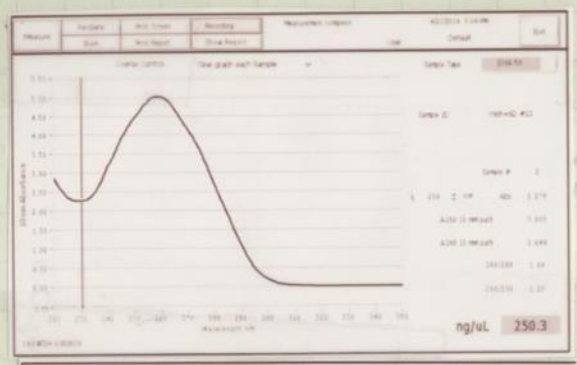
#7 - Negative 4/18/14

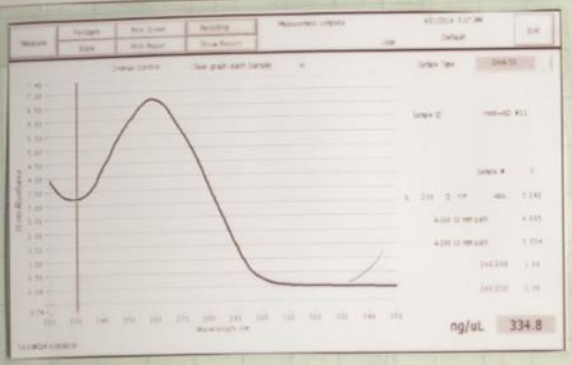
Try Again !!

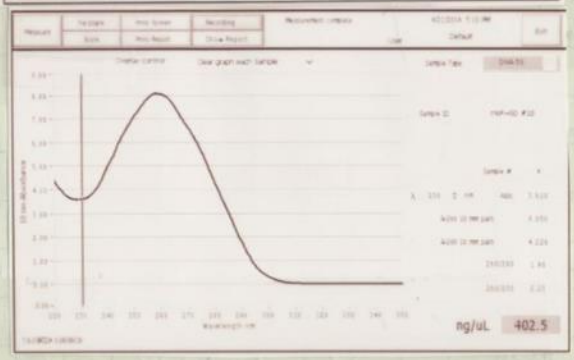
DNA Extraction of mlsR + 6D (2nd round)

4/21/









* All extractions look good. Now Digest and run a gel to screen for inserts.

Digest of mlsR+6D

4/22

mlsR+6D #8 $500\,ng \left| \frac{1\,uL}{407\,ng} \right. = 1.23\,uL$

mlsR+6D #10 $500\,ng \left| \frac{1\,uL}{250.3\,ng} \right. = 2.00\,uL$

mlsR+6D #11 $500\,ng \left| \frac{1\,uL}{334.8\,ng} \right. = 1.49\,uL$

mlsR+6D #18 $500\,ng \left| \frac{1\,uL}{402.5\,ng} \right. = 1.24\,uL$

Sample	EcoRI	PstI	10x Buffer	BSA	DNA
mlsR+6D #8	1 µL	1 µL	2 µL	1 µL	1.23 µL
mlsR+6D #10	1 µL	1 µL	2 µL	1 µL	2.00 µL
mlsR+6D #11	1 µL	1 µL	2 µL	1 µL	1.49 µL
mlsR+6D #18	1 µL	1 µL	2 µL	1 µL	1.24 µL
mlsR #24	1 µL	1 µL	2 µL	1 µL	1.10 µL

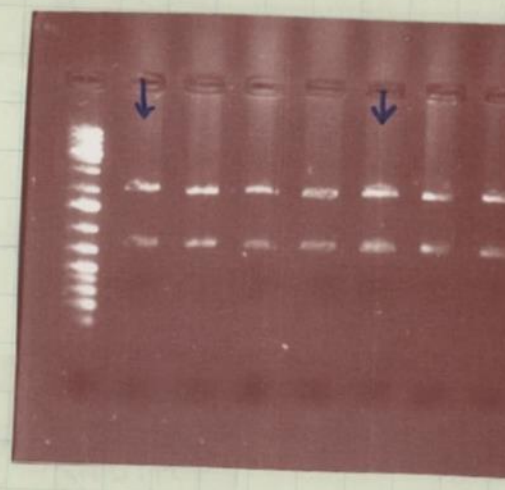
- Place in heat bath @ 42°C for 45 min.
- Place on heat block @ 80°C for 15 min.

4/22/14 Gel of mlsR + 6D

* Digest mlsR #24 to compare. Use tabl

Row 1

Lane 1 - 1kb Plus
Lane 2 - mlsR+6D #8
Lane 3 - mlsR #24
Lane 4 - mlsR+6D #10
Lane 5 - mlsR #24
Lane 6 - mlsR+6D #11
Lane 7 - mlsR #24
Lane 8 - mlsR+6D #18



* Send mlsR+6D #11 and mlsR+6D #8 for
Sequencing

Sent mlsR+6D for sequencing

4/

Sent samples mlsR+6D #11 and mlsR+6D #8 for sequencing. Send 5 μL of each.

Results of sequencing

mlsR+6D #11 – Negative 4/25/14

mlsR+6D #8 – Negative 4/25/14

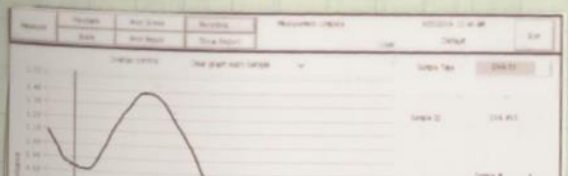
* Redo 3A.
* 5 promoters (confirmed) were plated from freezer stocks. 4/2

Plasmid Extraction of Promoters

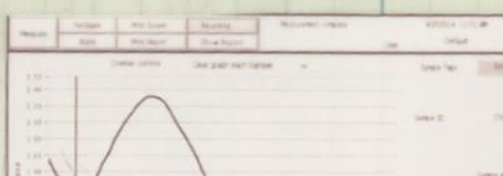
4/2

20E #14

↳ Not usable. Reextract DNA from plate.



Good →



4/28/14 Sequencing Adh1, Adh6 + Ter

Samples of Adh1 from 8/7/13 were found the stocks containing the insert but not sequenced. The same is true for Adh6 from 9/11/13.

Adh6 #1 $500\,ng \left| \frac{1\,\mu L}{755.6\,ng} \right. = 0.662\,\mu L$

Send 4 µL

Ter #4 $500\,ng \left| \frac{1\,\mu L}{855.6\,ng} \right. = 0.584\,\mu L$

Send 4 µL

Adh1 #24 $500\,ng \left| \frac{1\,\mu L}{261.6\,ng} \right. = 1.911\,\mu L$

Send 6 µL

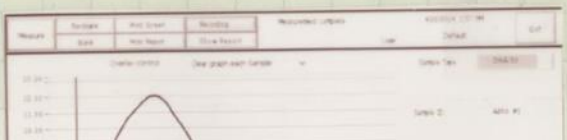
Results of sequencing

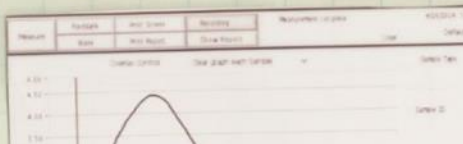
Adh6 #1— Not sure 4/30/14

Ter #4— Not sure 4/30/14

Adh1 #24— 4/30/14

* Adh6 #1 + Ter #4 was diluted to 600 and 720 ng/µL respectively.





* Reorder new V_{F2} + V_R primers. When sent for sequencing, larger genes are not fully sequenced.

Digest for mlsR + Terminator 3A assembly #2 5/12

500 ng	Upstream part (mlsR)
1 µL	Eco RI
1 µL	Spe I
5 µL	10x Buffer
1 µL	BSA
add to make 50 µL Total	PCR H_2O

500 ng	Downstream part (Terminator)
1 µL	Xba I
1 µL	Pst I
5 µL	10 x Buffer
1 µL	BSA
add to make 50 µL Total	PCR H_2O

500ng	Destination Plasmid (psB1K3)
1 µL	Eco RI
1 µL	Pst I
5 µL	10x Buffer
1 µL	BSA
add to make 50 µL Total	PCR H_2O

* 3A of both mlsR + 4P and mlsR + 6D are the target parts to be made in this 3A assembly.

mlsR #24 — $\frac{500\,ng}{455.1\,ng} \mid \frac{1\,\mu L}{}$ = 1.10 µL in
of H_2O.

4P #10 — $\frac{500\,ng}{299.8\,ng} \mid \frac{1\,\mu L}{}$ = 1.67 µL in
of H_2O.

6D #16 — $\frac{500\,ng}{194.2\,ng} \mid \frac{1\,\mu L}{}$ = 2.57 µL in
of H_2O.

pSB1K3 #1 — $\frac{500\,ng}{175.2\,ng} \mid \frac{1\,\mu L}{}$ = 2.85 µL in
of H_2O.

* Place in heatbath for 45 min at 42°C.
* Heatkill enzymes on heatblock for 15 min at

5/12/14 Ligation of 2nd mlsR + T 3A

4 µL of upstream part
4 µL of downstream part
4 µL of destination backbone
4 µL of 10x T4 ligase buffer
1 µL of T4 ligase
22 µL of H_2O

* Incubate on bench top for 20 min.
* Ready to transform.

Transformation of 2nd mlsR + T 3A

5/12

- Transform 4 µL of Ligation product into 100 µL of competent cells for each transformation.
- Keep on ice for 30 min.
- Heat shock in waterbath for 60 sec at 42°C One set in water bath for 60 sec and the other set placed in water bath for 105 seconds.
- Place back on ice for 5 min.
- add 600 µL of psi broth
- Incubate at 37°C for 2 hours at 200 rpms.
- Then plate using a dilution of 1:1 and 1:10.

* It is expected that a longer heatshock will increase transformation efficiency.

AquaPlasmid Extraction Procedure Obtained

5/20

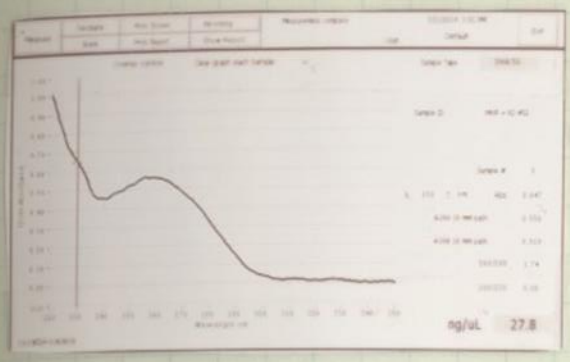
- ~~Pippe~~ Pipette 200 µL of aquaplasmid solution into a 1.5 mL centrifuge tube.
- Add a loopful of cells to solution
- Immediately touch-vortex until homoginized.
- Incubate at room temperature on bench-top for 10 min to lyse cells.
- Incubate crude lysate at -20°C for 10 min on ice to induce precipitation.

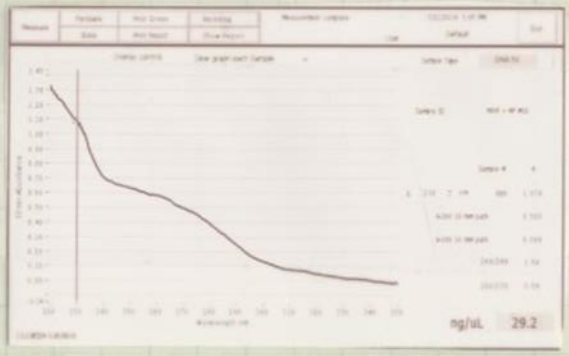
- Transfer clear lysate to a clean 1.5 mL cen tube.
  * Be careful to ONLY get supernatant a NONE of the pellet.
- Add 0.5 volume of isopropanol to lysate.
  * Do not use more than 0.7 vol for DNA precipitation. to avoid contamination.
- Touch vortex to mix well.
- Centrifuge @ 14,000 rpm for 5 min at 22°C pellet the ~~plasid~~ plasmid DNA.
- Flip tube forcefully a few times to discard supernatant.
- To rinse DNA pellet (nearly invisible; a lar DNA pellet indicates that the cell density exceeds capacity of aquaplasmid) overflo tube with 70% ethanol.
- Flip tube forcefully a few times and blot it paper towel to remove residual ethanol.
- Leave tube upside down on a paper towel 5 min to air-dry DNA pellet.
- Add 50 µL of DI water to DNA pellet.
- Vortex for 30 sec and incubate at 22°C for min to fully solubilize DNA
- Centrifuge at 14,000 rpm for 3 min to pellet insoluble (nearly invisible)

AquaPlasmid Extraction of mlsR + T

5/21/

* Using the Aquaplasmid Extraction protocol on mlsR + T. (pg.37)



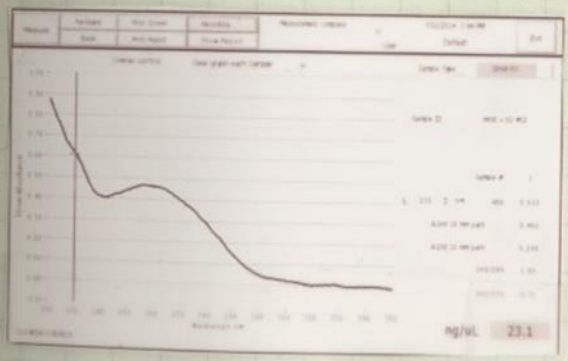


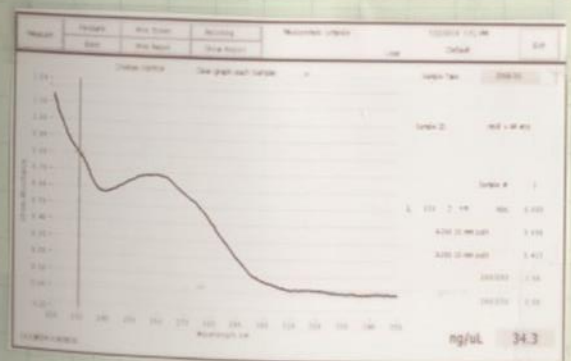
No Good Revise Protocol

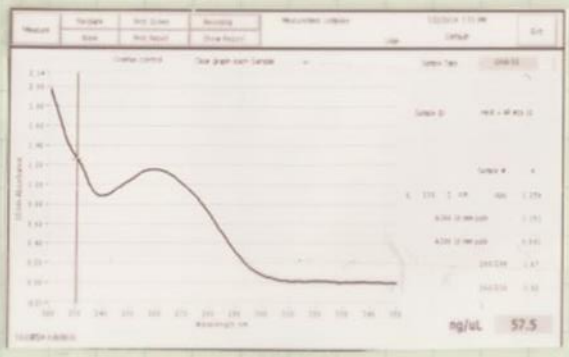
AquaPlasmid ~~Extraction~~ of mlsR+T Round 2

5/22

* Using the AquaPlasmid Extraction protocol on mlsR+T, ~~replace 14,000 rpm with 10,000rpm.~~





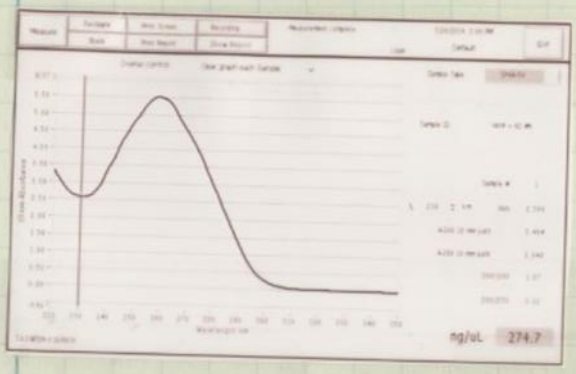


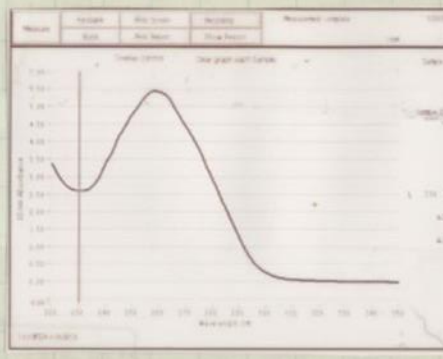
5/28/14

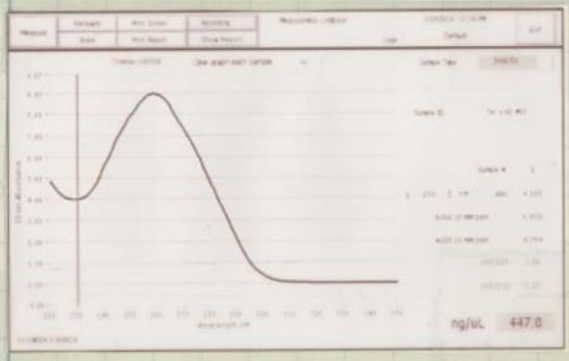
Plasmid Extraction of 20E, mlsR+T, Tet+T, and K

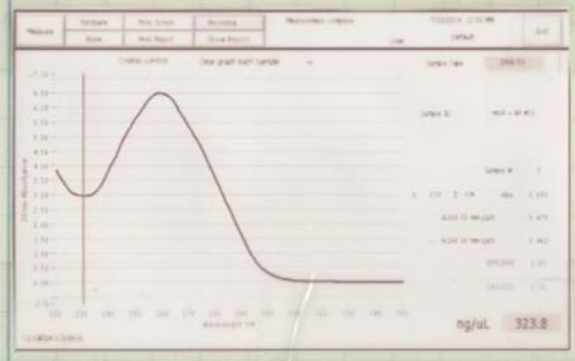
Using the QIA prep Spin Miniprep Kit (250
following the provided instruction, the following
were obtained.

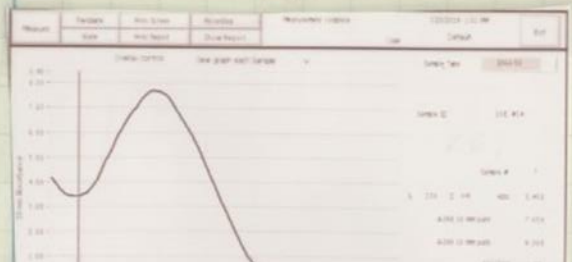
* We discovered that the PCR Purification
will also work for Plasmid Extraction K

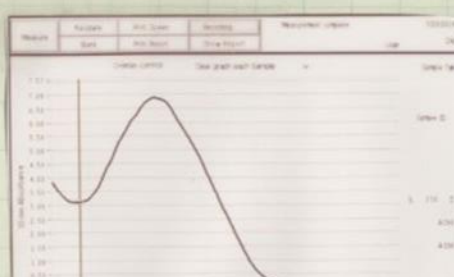


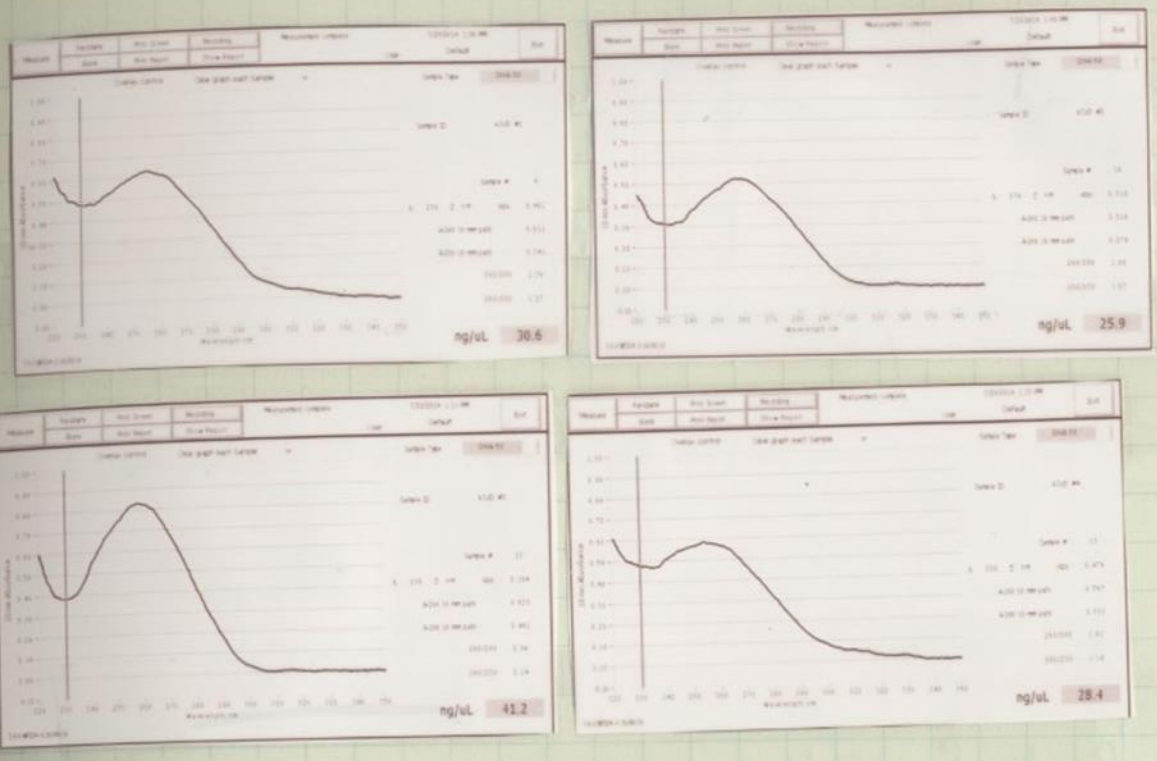












Digest of 20E #11, 20E #14, 20A #15 + 23B #3

5/2

- It is necessary to screen for an insert in our promoter plasmid to ensure that the parts we need are actually there.

20A #15 $500\,ng \left| \frac{1\,\mu L}{69\,ng} \right. = 7.25\,\mu L$ in $34.75\,\mu L$ of PCR H_2O

20E #11 $500\,ng \left| \frac{1\,\mu L}{390.6\,ng} \right. = 1.28\,\mu L$ in $40.72\,\mu L$ of PCR H_2O

20E #14 $500\,ng \left| \frac{1\,\mu L}{383.0\,ng} \right. = 1.31\,\mu L$ in $40.69\,\mu L$ of PCR H_2O

500ng	Promoter	All in 1 tube (50μL Rxn)
1 μL	EcoRI	
1 μL	Pst I	
5 μL	10x Buffer	
1 μL	BSA	
Add to make 50μL Total	PCR H_2O	

– 1 per each promoter (20A #15, 20E #11, 20E #14

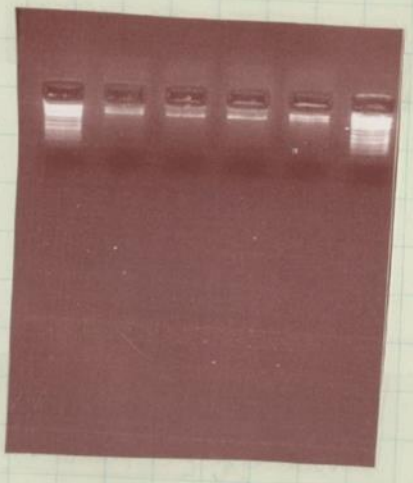
- Place in heat bath for 45 min at 42°C.
- Immediately place on heat block for 15 min at
- Ready for gel.

5/29/14 Gel of Promoters

* Make a 2% agarose gel in order ~~to see the~~ to the promoters' bands.

Row 1

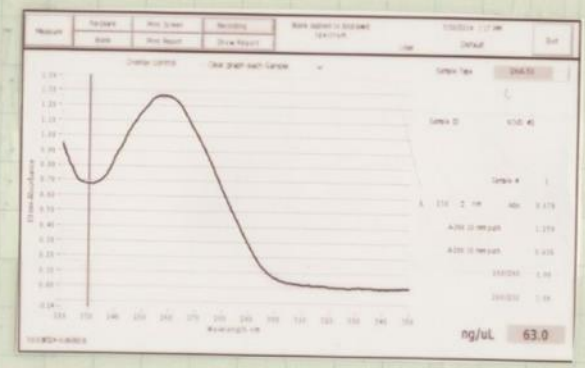
Lane 1 – 1kb Plus
Lane 2 – 20A #15
Lane 3 – 20E #11
Lane 4 – 20E #14
Lane 5 – 23B # 3
Lane 6 – 1kb Plus
Lane 7 – Empty
Lane 8 – Empty



Plasmid Extraction Via Extraction Kit of KIVD

5/

Used QIAprep Spin Miniprep Kit (250) on KIVD #1 + KIVD #2.



Digest of KIVD for gel and 3A assembly

6/

500ng	KIVD
1 μL	EcoRI
1 μL	PstI
5 μL	10x Buffer
1 μL	BSA
Add to make 50 μL Total	PCR H_2O

KIVD #1 $500\,ng \left| \frac{1\,\mu L}{77.7\,ng} \right. = 6.44\,\mu L$ in $35.56\,\mu L$ of PCR H_2O

KIVD #2 $500\,ng \left| \frac{1\,\mu L}{63\,ng} \right. = 7.94\,\mu L$ in $34.06\,\mu L$ of PCR H_2O

* Place in heat bath for 45 min at 42°C.

* Pl

6/2/14 Digest of mlsR+T and Ter+T

500ng	mlsR+T
1μL	Xba I
1μL	Pst I
5μL	10x Buffer
1μL	BSA
add to make 50μL Total	PCR H_2O

500ng	Ter+T
1μL	Xba I
1μL	Pst I
5μL	10x Buffer
1μL	BSA
add to make 50μL Total	PCR H_2O

mlsR+6D #9 $500ng \left| \frac{1\mu L}{274.7ng} \right. = 1.82$ μL of H_2

mlsR+6D #12 $500ng \left| \frac{1\mu L}{270.7ng} \right. = 1.85$ μL of H_2

mlsR+4P #13 $500ng \left| \frac{1\mu L}{323.8ng} \right. = 1.54$ μL of H

mlsR+4P #16 $500ng \left| \frac{1\mu L}{293.8ng} \right. = 1.70$ μL of

Ter+4P #5 $500ng \left| \frac{1\mu L}{342.3ng} \right. = 1.46$ μL of

Ter+6D #13 $500ng \left| \frac{1\mu L}{447ng} \right. = 1.12$ μL of

* Also Digest Ter and mlsR to compare to the 3A s.

Ter #4 $500ng \left| \frac{1\mu L}{855.6\,ng} \right. = 1\,\mu L$ in $41\,\mu L$ of H_2O.

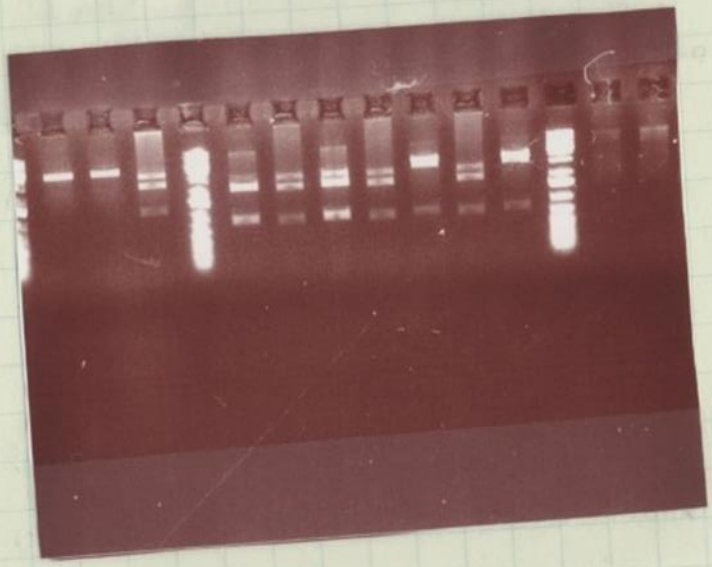
mlsR #24 $500ng \left| \frac{1\mu L}{455.1\,ng} \right. = 1.1\,\mu L$ in $40.9\,\mu L$ of H_2O.

6/

Gel of mlsR+T, Ter+T, and KIVD

Row 1

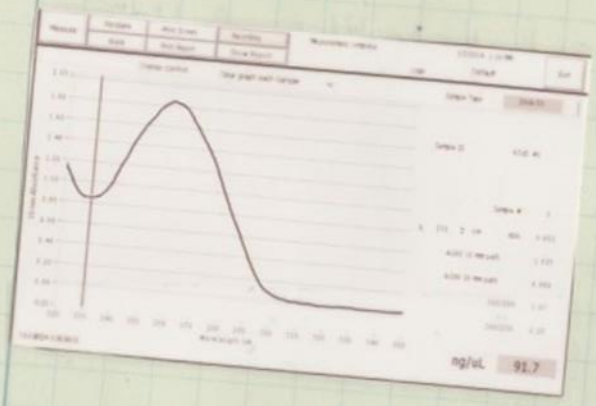
Lane 1 - 1 kb Plus
Lane 2 - Ter + 4P #5
Lane 3 - ~~Ter #4~~ Ter + 6D #13
Lane 4 - ~~Ter + 6D #13~~ Ter #4
Lane 5 - 1 kb Plus
Lane 6 - mlsR + 6D #9
Lane 7 - mlsR #24
Lane 8 - mlsR + 6D #12
Lane 9 - mlsR #24
Lane 10 - mlsR + 4P #13
Lane 11 - mlsR #24
Lane 12 - mlsR + 4P #16
Lane 13 - 1 kb Plus
Lane 14 - KIVD #1
Lane 15 - KIVD #2
Lane 16 - 1 kb Plus



- No inserts found for Ter + T or KIVD.
  - Extract Plasmids from new libraries.

6/3/14 Plasmid Extraction of KIVD + Ter+4P

Used the Kit for this extraction



6/3/14 Digest of KIVD and Ter+T

500 ng	KIVD #1
1 µL	Xba I
1 µL	Pst I
5 µL	10x Buffer
1 µL	BSA
add to make 50 µL Total	PCR H_2O

500 ng	Ter+T (Ter+4P #1)
1 µL	Xba I
1 µL	Pst I
5 µL	10x Buffer
1 µL	BSA
add to make 50 µL Total	PCR H_2O

KIVD #1 500 ng | $\frac{1\,\mu L}{91.7}$ = 5.45 µL in 36.55 µL

~~- Place on heatblock for 45 min at ~~

- Place in heatbath for 45 min at 42°C
- Place on heatblock for 15 min at 80°C

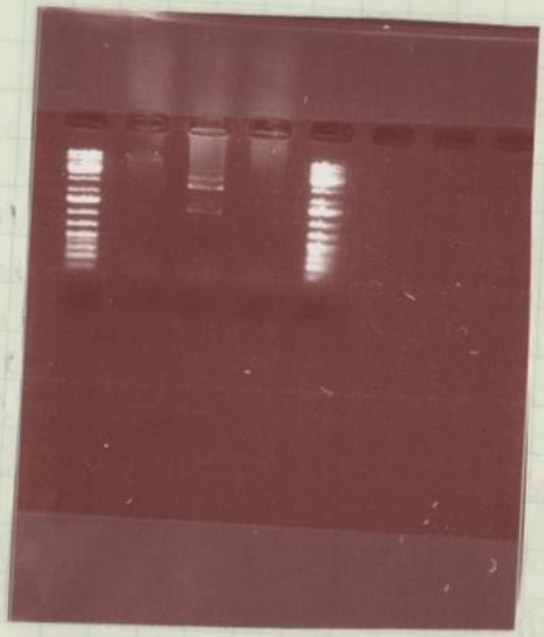
* Also digest ~~mtsR#24~~ using the calculations on page 45.
  ↑ Ter #4
* The Digest of Ter is to be compared to Ter + 4P #1 to verify correct insert size.

Gel of KIVD and Ter+4P

6/3

Row 1

Lane 1 - 1kb Plus
Lane 2 - Ter +4P #1
Lane 3 - Ter #4
Lane 4 - KIVD #1
Lane 5 - 1kb Plus
Lane 6 - Empty
Lane 7 - Empty
Lane 8 - Empty

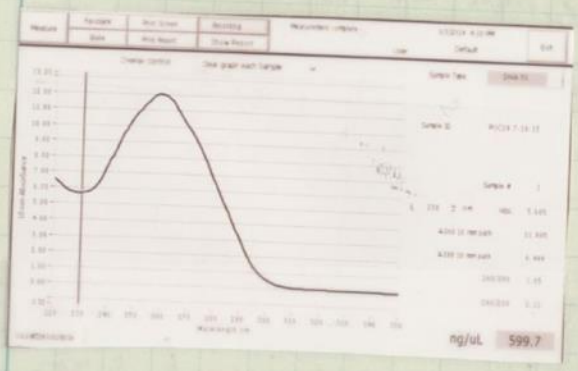


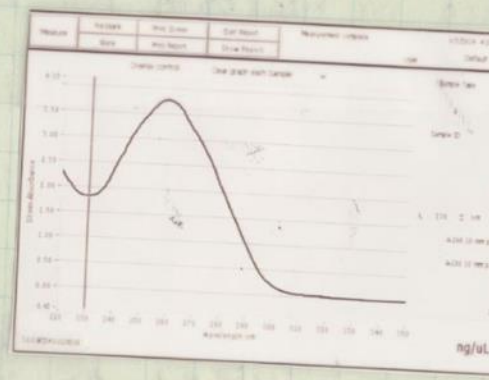
* Search freezer for original KIVD samples.

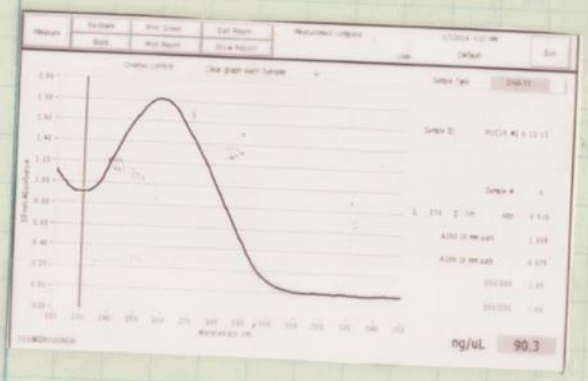
6/3/14

Digest of PUC19 Samples

- PUC19 Samples from last year were







* PUC19 contains K

500ng	PUC19
1 µL	Xba I
1 µL	Pst I
5 µL	10x Buffer
1 µL	BSA
add to make 50µL Total	PCR H_2O

PUC 19 - 7/18/13 Sample

$$500\,ng \left| \frac{1\,\mu L}{599.7\,ng} \right. = 1\,\mu L \text{ in } 41\,\mu L \text{ of } H_2$$

PUC19 - 6/12/13 #1 Sample

$$500\,ng \left| \frac{1\,\mu L}{187.6\,ng} \right. = 2.67\,\mu L \text{ in } 39.33\,\mu L \text{ of}$$

- Place in heatbath for 45 min at 42°C.
- Place on heatblock for 15 min at 80°C.

Gel of PUC19 6/3

Row 1

Lane 1 - 1 kb Plus
Lane 2 - PUC19 7/18/13
Lane 3 - PUC19 6/12/13 #1
Lane 4 - PUC19 6/12/13 #2
Lane 5 - Empty
Lane 6 - Empty
Lane 7 - Empty
Lane 8 - Empty

Digest of Ori+P_{ptb-} and mlsR+T for 3A assembly 6/4

500 ng	Upstream Part (Ori+P_{ptb-} #3)
1 µL	~~Downstream Part (mlsR+T)~~ EcoRI
1 µL	~~EcoRI~~ Spe I
5 µL	10x Buffer
1 µL	BSA
add to make 50 µL Total	PCR H_2O

500ng	Destination Plasmid (pSB1C
1 µL	EcoRI
1 µL	PstI
5 µL	10x Buffer
1 µL	BSA
add to make 50µL Total	PCR H_2O

Ori + P_{tet} - #3 — 500 ng | 1 µL / 112 ng = 4.46 µL in 3 of H_2O

mlsR + 4P #13 — 500 ng | 1 µL / 323.8 ng = 1.54 µL in 4 of H_2O

mlsR + 6D #12 — 500 ng | 1 µL / 270.7 ng = 1.85 µL in 4 of H_2O

pSB1C3 #1 — 500 ng | 1 µL / 232 ng = 2.16 µL in 3 of H_2O

* Place in heatbath for 45 min at 42°C.

* Place on heat block for 15 min at 80°C

6/4/13 Sent mlsR+6D #12 and mlsR+4P#13 for sequenci

- 2 samples of mlsR+T were sent to seque

Results of sequencing

Ligation of Ori + P_{ptb-} + mlsR + T

6/4

4 µL of Ori + P_{ptb-} #3
4 µL of mlsR + 6D #12
4 µL of pSB1C3 #1
4 µL of 10x T4 ligase buffer
1 µL of T4 ligase
22 µL of PCR H_2O

} x2

4 µL of Ori + P_{ptb-} #3
4 µL of mlsR + 4P #13
4 µL of pSB1C3 #1
4 µL of T4 10x ligase buffer
1 µL of T4 ligase
22 µL of PCR H_2O

} x2

* Use 4 µL of each digested product.

- Incubate on bench top for 20 min.
- Ready to Transform.

Transformation of ori + P_{ptb-} + mlsR + T

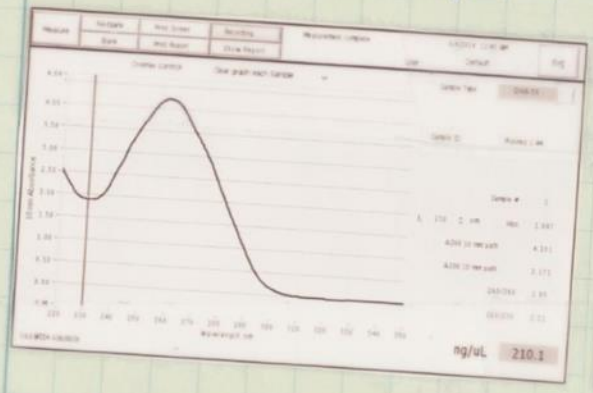
6/

- Transform 4 µL of ligation product into 100 µL tubes of competent cells.
    * 8 transformations total were conducted.
    * Ori + P_{ptb-} + mlsR + 4P will be known as Plasmid #1 (P1).
        - Ori + P_{ptb-} + mlsR + 6D will be known as

- Add 600 µL of psi broth to each transformat
- Incubate at 37°C for 2 hours at 200 rpms
- Make 1:10 dilutions. (90 µL psi broth and 10 µL
- Plate the dilutions appropriately.
- Incubate plates at 37°C for 24-48 hours.

6/9/14 DNA Extraction of Plasmid #1 + #2

- Extraction was performed with the kit.



6/9/14 Digest of Plasmid #1 + #2

500 ng	Plasmid
1 µL	Pst I
1 µL	Xba I
5 µL	

Plasmid 1 #4 $500\,ng \left| \frac{1\,\mu L}{210.1\,ng} \right. = 2.4\,\mu L$ in $39.6\,\mu L$ of H_2O

Plasmid 2 #2 $500\,ng \left| \frac{1\,\mu L}{198.8\,ng} \right. = 2.5\,\mu L$ in $39.5\,\mu L$ of H_2O.

— Place in heatbath for 45 min at 42°C.

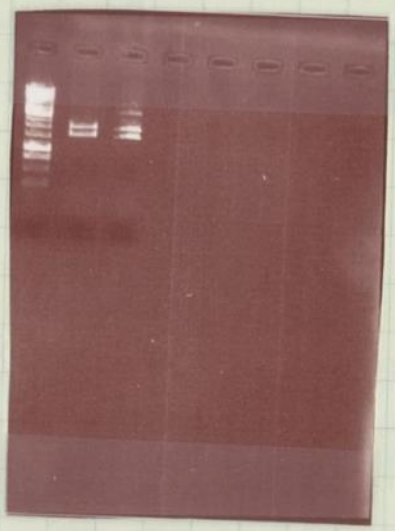
— Place on heatblock for 15 min at 80°C.

Gel of Plasmid 1 and Plasmid 2

6/

Row 1

Lane 1 – 1kb [redacted]
Lane 2 – Plasmid 1 #4
Lane 3 – Plasmid 2 #2
Lane 4 – Empty
Lane 5 – Empty
Lane 6 – Empty
Lane 7 – Empty
Lane 8 – Empty



* Used the 1 kb generuler ladder. Insert size is as expected.

– Possible success –

6/9/14 **Shuttle Vector Sent for Sequencing**

Samples sent for sequencing are:

Plasmid 1 #4 – Not sure 6/10/14

Plasmid 2 #2 – Not sure 6/10/14

* The beginning and end of the sequences are th
the middle was not sequenced. *

6/9/14 **Growing up BktB from registry**

- For future reference the registry part # for
is:

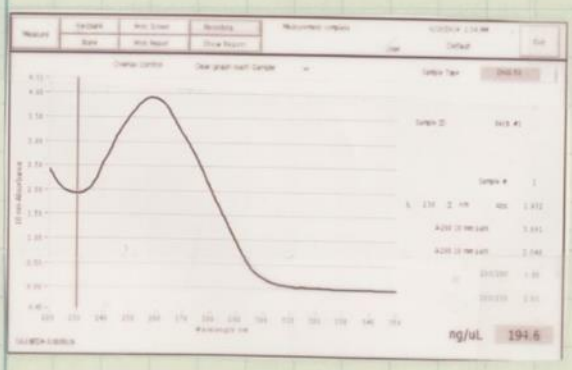
BBa_K759004

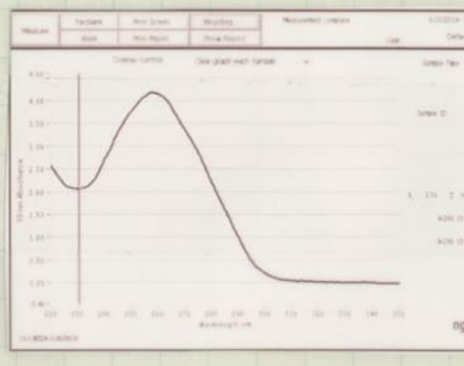
- BktB has been streaked onto plate and will
libraries made from the colonies.

BBa_K759004
pSB1C3
5/21/2014

6/10/14 **DNA Extraction of BktB**

- Used the kit.





Digest of BktB

6/24

500 ng	BktB
1 µL	EcoRI
1 µL	Pst I
5 µL	10x Buffer
1 µL	BSA
add to make 50 µL total	PCR H_2O

BktB #1 500 ng | 1 µL / 194.6 ng = 2.6 µL in 39.4 µL of H_2O.

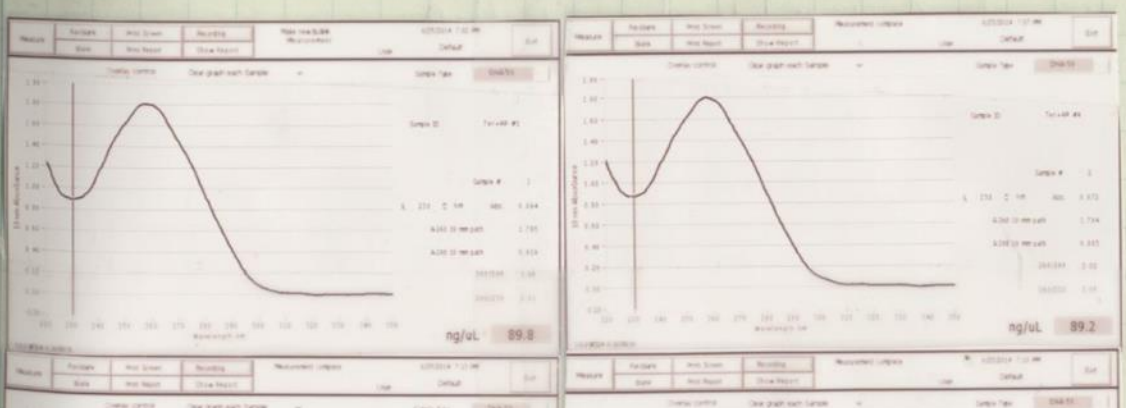
BktB #2 500 ng | 1 µL / 207.5 ng = 2.4 µL in 39.6 µL of H_2O.

* The gel and digest was performed by Ian Pratt

- The results indicate that the digest was unsuccessful.
- I will personally attempt the digest and gel of BktB at a later time.

DNA Extraction of Ter + T

6/25



6/26/14

Digest of BktB and Ter+T

BktB #1 — 500 ng | 1 µL / 194.6 ng = 2.57 µL in 3
H_2O.

BktB #2 — 500 ng | 1 µL / 207.5 ng = 2.41 µL in 3
H_2O.

Ter #4 — 500 ng | 1 µL / 855.1 ng ≈ 1 µL in 41
H_2O.

Ter+4P #1 — 500 ng | 1 µL / 89.8 ng = 5.57 µL in 3
H_2O.

Ter+4P #4 — 500 ng | 1 µL / 89.2 ng = 5.61 µL in 36
H_2O.

Ter+6D #1 — 500 ng | 1 µL / 133.9 ng = 3.73 µL in 38
H_2O.

Ter+6D #2 — 500 ng | 1 µL / 78.4 ng = 6.38 µL in 35
H_2O.

500 ng	Sample DNA
1 µL	Eco RI
1 µL	PstI
5 µL	10x FD Buffer
1 µL	BSA
add to make 50 µL total	PCR H_2O

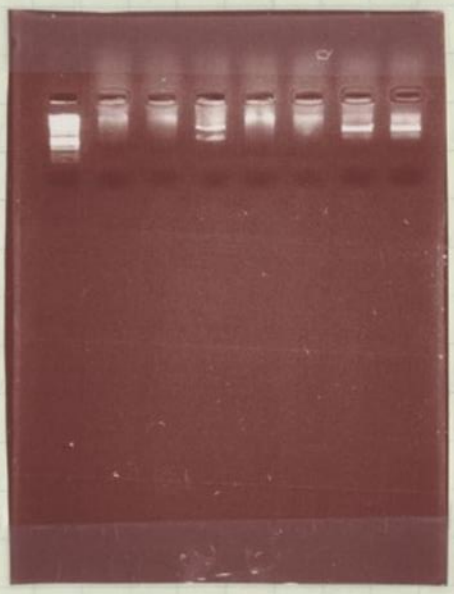
* Place in waterbath for 45 min at 42°C.
* Place on heat block for 15 min at 80°C.

Gel of BktB and Ter+T

6/

Row1

Lane1 – 1 kb
Lane2 – Ter +4P #1
Lane3 – Ter +4P #4
Lane4 – Ter #4
Lane5 – Ter +6D #1
Lane6 – Ter +6D #2
Lane7 – BktB #1
Lane8 – BKtB #2



* BktB Both seem to be in the plasmid. Next step is to Send them for sequencing.

* Ter + T inserts are not present in these samples. Reselect colonies from the libraries. (maybe perform a colony PCR).

* A colony PCR could screen for inserts in many samples all at once. This would save us some time.

Digest of Promoters, Terminators, KIVD, Ter, and BktB

7

500 ng	DNA [redacted] (Upstream part)
1 µL	EcoR1
1 µL	Spe 1
5 µL	10 x FD Buffer
1 µL	BSA
add to make 50 µL Total	PCR H_2O

500 ng	Destination Plasmid
1 µL	Eco R1
1 µL	Pst 1
5 µL	10x FD Buffer
1 µL	BSA
add to make 50 µL total	PCR H_2O.

Upstream Parts	Downstream Parts	Destination Pl
- Promoters	- Terminators	- pSB1K3
• 20A, 20E, 23B	• 4P + 6D	- pSB1T3
- KIVD	- BktB	
- Ter		

Target Assemblies

Promoters + BktB + pSB1T3
KIVD + Terminators + pSB1K3
Ter + Terminators + pSB1K3

20A #15 500 ng $\left|\frac{1\ \mu L}{353.3\ ng}\right.$ = 1.42 µL in 40.58 µL

20E #14 500 ng $\left|\frac{1\ \mu L}{198.7\ ng}\right.$ = 2.52 µL in 39.48 µL

23B #3 500 ng $\left|\frac{1\ \mu L}{405.8\ ng}\right.$ = 1.23 µL in 40.77 µL

KIVD #1 2,000 ng $\left|\frac{1\ \mu L}{599.7\ ng}\right.$ = 3.34 µL in 38.66 µL

4P#10 $500\,ng \left| \frac{1\,\mu L}{299.8\,ng} \right. = 1.67\,\mu L$ in $40.33\,\mu L$ of H_2O.

6D#16 $500\,ng \left| \frac{1\,\mu L}{194.2\,ng} \right. = 2.57\,\mu L$ in $39.43\,\mu L$ of H_2O.

pSB1K3#2 $500\,ng \left| \frac{1\,\mu L}{175.7\,ng} \right. = 2.85\,\mu L$ in $39.15\,\mu L$ of H_2O.

pSB1T3#2 $500\,ng \left| \frac{1\,\mu L}{142.5\,ng} \right. = 3.51\,\mu L$ in $38.49\,\mu L$ of H_2O.

- Place in heat bath for 45 min at 42°C.
- Place on heatblock for 15 min at 80°C.

Ligation of the three 3A assemblies 7/2

$4\,\mu L$ of digested BktB
$4\,\mu L$ of digested 20A#15
$4\,\mu L$ of digested pSB1T3
$4\,\mu L$ of 10x T4 Ligase Buffer
$1\,\mu L$ of T4 Ligase
$22\,\mu L$ of PCR H_2O

$4\,\mu L$ of digested BktB
$4\,\mu L$ of digested 20E#14
$4\,\mu L$ of digested pSB1T3
$4\,\mu L$ of 10x T4 Ligase Buffer
$1\,\mu L$ of T4 Ligase
$22\,\mu L$ of PCR H_2O

4 µL of digested KIVD #1
4 µL of digested 4P #10
4 µL of digested pSB1K3
4 µL of 10x T4 Ligase Buffer
1 µL of T4 Ligase
22 µL of PCR H_2O

4 µL of digested Ter #4
4 µL of digested 4P #10
4 µL of digested pSB1K3
4 µL of 10x T4 Ligase Buffer
1 µL of T4 Ligase
22 µL of PCR H_2O

4 µL of digested KIVD #1
4 µL of digested 6D #16
4 µL of digested pSB1K3
4 µL of 10x T4 Ligase Buffer
1 µL of T4 Ligase
22 µL of PCR H_2O

4 µL of digested Ter #4
4 µL of digested 6D #16
4 µL of digested pSB1K3
4 µL of 10x T4 Ligase Buffer
1 µL of T4 Ligase
22 µL of PCR H_2O

- Incubate on bench top for 20 min.
- Ready to transform.

Transformation of 3 3A Assemblies

- Add 4μL of ligation product into 100μL of Top10 ~~competen~~ competent cells.
- Place on ice for 30min.
- Place in heat bath at 42°C for 60 sec exactly.
- Place back on ice for 5 more minutes.
- Add 600μL of psi broth
- Place in the shaking incubator for 2 hours at 37°C at 200 rpms.
- Make 1:1 and 1:10 dilutions and plate them Accordingly.

Results: KIVD + T and Ter + T grew a good amount of white colonies.

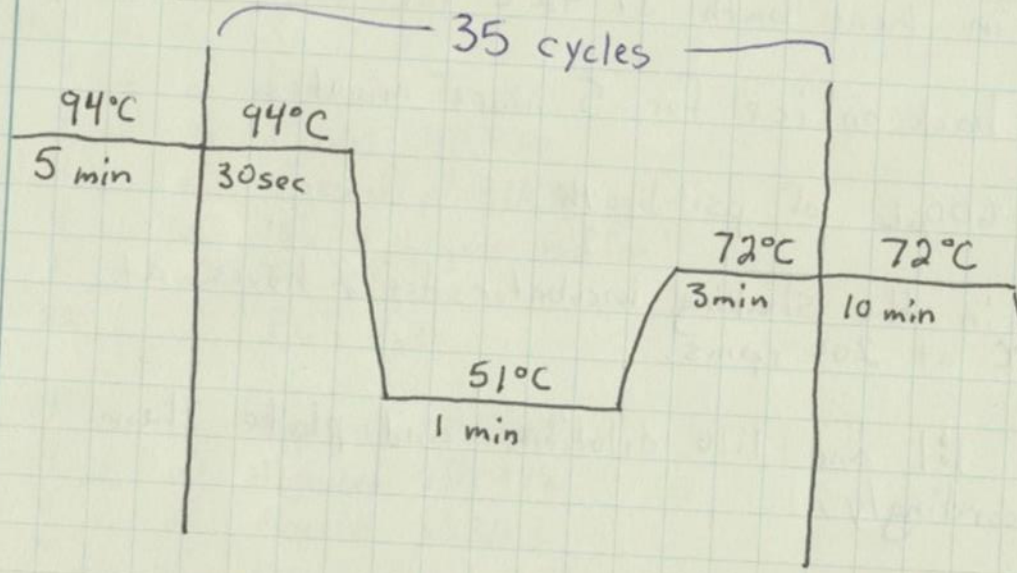
Transformation of Adh6

- Add 4μL of Adh6 into 100μL of Top10 competent cells
- Place on ice for 30 min
- Place in heatbath for 60 seconds at 42°C
- Place back on ice for 5 more minutes.

7/17/14 Colony PCR of Ter and KIVD for freezer

30 µL
x 30 rxns
900 µL Total

450 µL MM
2 µL Fwrd Primer
2 µL Rvrs Primer
446 µL PCR H_2O
900 µL Total
} x 2

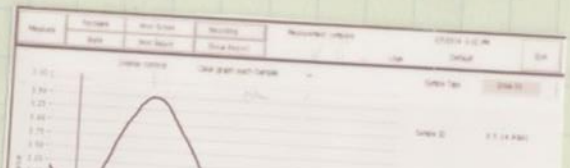


* Thermocycler [redacted] failed during the 26th cycle PCR is ruined.

- Note-to-self - Use doctor Wawrik's thermocycler for the next Colony F

8/5/14 DNA Extraction of PAN1

Using the QIAGEN miniprep Kit, PAN1 Plas extracted.



Confirming Methylation

8/

Erin W. Electroporated P1 and P2 into electrocompetent cells of DH5α *E. coli* containing the PAN1 methylating plasmid (8/4/14). The plates contained good growth with many colonies. A gel should be run comparing P1, P2, PAN1 and the plasmid extraction of the transformed cells.

Row 1

Lane 1 - 1 kb ladder
Lane 2 - P1
Lane 3 - P2
Lane 4 - Electroporated sample #1
Lane 5 - PAN1
Lane 6 - 1 kb ladder
Lane 7 - Empty
Lane 8 - Empty

* Placing the electroporated sample in between the shuttle vectors and PAN1 should allow us to compare the plasmids in the sample to the plasmids we should expect to see.

* Next extract DNA from the electroporated samples.

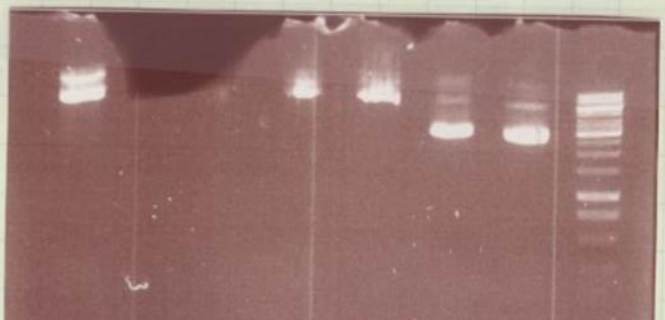
* When running the gel use ~~500ng~~ 5μL of DNA for each well.

Methylation Confirmation Gel

8/

Row 1

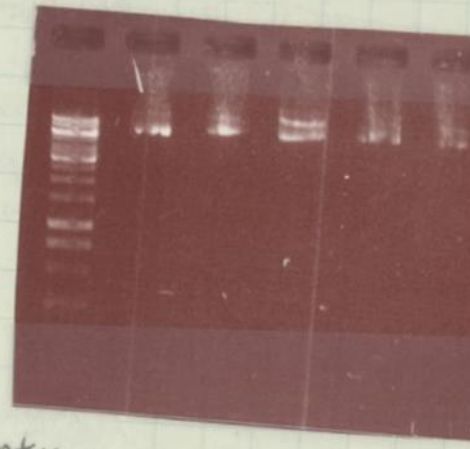
Lane 1 - Sample 1
Lane 2 - Sample 2
Lane 3 - Sample 3
Lane 4 - Sample 4
Lane 5 - PAN1
Lane 6 - P1



8/20/14

Methylation Gel Continued

Row 2 (Gel 2)



Lane 1- Ladder 1 kb
Lane 2- PAN1
Lane 3- Sample 1
Lane 4- Sample 2
Lane 5- Sample 3
Lane 6- Sample 4
Lane 7- Ladder 1kb
Lane 8- ~~[illegible]~~ Empty

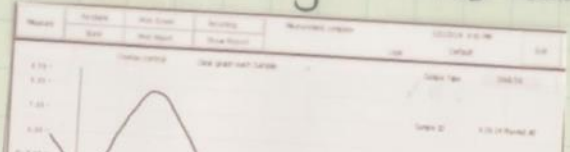
* These gels were run to determine
not the P1 or P2 plasmids were
presence of the PAN1 methlating plasm
The resulting smears on the gel indicate
undigested plasmid form is not suitab
electrophoresis.
∴ The next steps to be taken should
~~determinig~~ determining if PAN1
Eco R1 restriction site for dig
- Then Digest the ~~plasit~~ plas
perform another gel.
* Results should be much cle

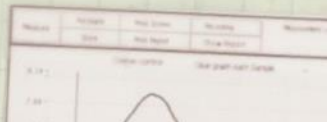
- Also we need more P1 and P2
- DNA Extraction is needed.

8/21/14

Plasmid Extraction of P1 and P2

Using the Thermo Scientific Plasmid Extrac
the following results were obtained.





Transformation of P1 + P2 into T3 (Digest)

9/

500 ng (2.8 µL)	pSB1T3 178.7 ng/µL
1 µL	Eco R1
1 µL	Pst I
2 µL	10x Digest Buffer
1 µL	BSA
add to make 20 µL total	PCR H_2O (12.2 µL)

500 ng (1.35 µL)	P1 370.9 ng/µL
1 µL	Eco R1
1 µL	Pst 1
2 µL	10x Digest Buffer
1 µL	BSA
add to make 20 µL total	PCR H_2O (13.65 µL)

500 ng (1.28 ≈ 1.29 µL)	P2 388.4 ng/µL
1 µL	EcoR1
1 µL	Pst1
2 µL	10x Digest Buffer
1 µL	BSA
add to make 20 µL Total	PCR H_2O (13.71 µL)

- Place in heatbath for 45 min at 42°C.
- Place on heatblock for 15 min at 80°C

9/24/14 Ligation of P1 + P2 into pSB1T3

10 µL pSB1T3
10 µL P1
4 µL 10x T4 ligase buffer
1 µL T4 Ligase
15 µL PCR H_2O

10 µL pSB1T3
10 µL P2
4 µL 10x T4 ligase buffer
1 µL T4 Ligase
15 µL PCR H_2O

- Let sit on bench top for 20 mins.

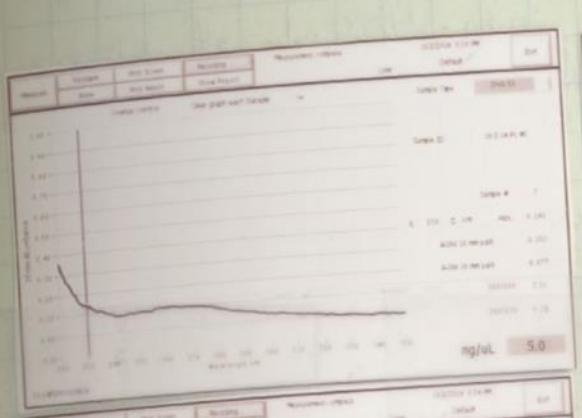
9/24/14 Transformation of P1 + P2 into pSB1

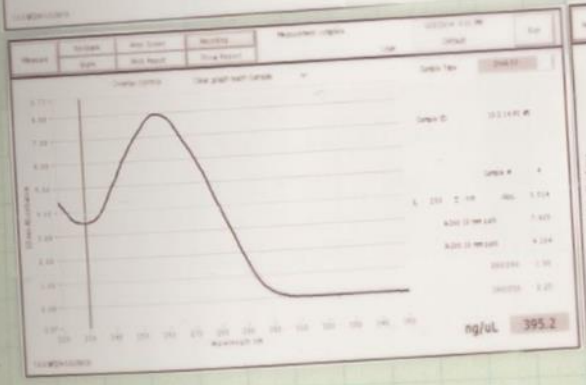
- Add 4 µL of ligation product into thawed competant cells.
- Place on ice for 30 mins.
- Place in heatbath for 60 seconds at
- Place back on ice for an additional
- Add 600 µL of 2XYT broth (psi broth available.)
- Incubate at 37°C for 2 hours at 20

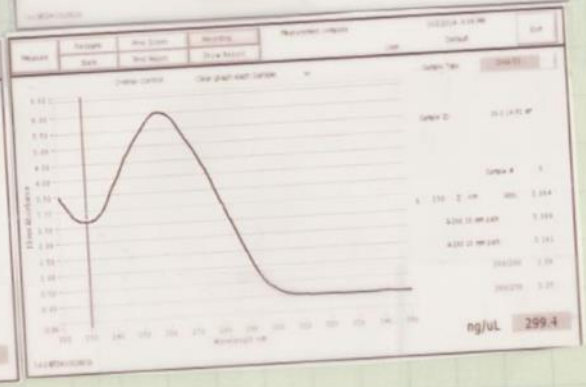
Plasmid Extraction of P1 + P2 in pSB1T3

10/

Using the Thermoscientific Plasmid Extraction Kit, the following results were obtained







10/8/14

Electroporation of PANI *E. coli* with P1

- Thaw DH5α cells (PANI) in ice from the freezer.
- Add 500 ng of DNA into 400 µL of e competent cells and let sit in ice fo 5 min.

  * Keep cuvette on ice at all times.
- Add the DNA/cells mix into the cu (still on ice) and ensure the metal pla are covered and that there are bubbles in between the metal plates.
- Let sit in ice for ~1 minute.
- Take cuvette out of the ice and dry outside with a Kim wipe.
- Place in the electroporator slot and slide all the way into the machine. (Ensure the plates are oriented in the corr direction.).
- Run electroporator at 2500 Volts (Setting P
- Immediately remove the cuvette and add of Psi broth, and gently mix.
- Immediately place contents into 1.5 mL tube and put on ice. (5 minutes).
- Incubate at 37°C for 2 hours at 200
- Plate 1:1, 1:10, 1:100 (and maybe 1:1,000) dilution

10/8

Results of Electroporation of P1 into PAN I

Electroporation attempt

#1	act 370V set 2500V	654 ms	–	Failure
#2	act 380V set 2500V	654 ms	–	Failure
#3	act 480 V set 2500 V	654 ms	–	Failure
#4	act 2450 V set 2500 V	5.9 ms	–	Success with water as control

* Ideal voltage = close to 2500V and close to 5 ms as possible *

#5	act 570 V set 2500 V	654 ms	–	Failure
#6	act 550 V set 2500 V	654 ms	–	Failure
#7	act 2410 set 2500 V	5.0 ms	–	Success

- #1–6 (with the exception of #4) were using P1KM1 plasmid electrocompetent cells and P1 #3 in (P1 is our Shuttle vector) electroporation.
- I realized we were using the wrong cells.
- #7 used the PAN1 electrocompetent cells and 600ng of P1 #7 in Tetracycline.

10/9/14

Results of E.coli Electroporation

The 7th attempt at electroporation seems to a success.

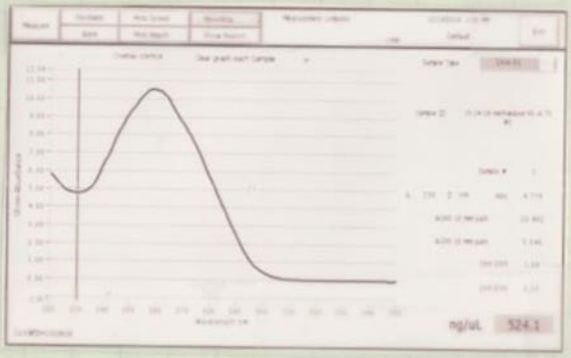
- The 1:1 had too many colonies to count.
- The 1:10 had +200 colonies.
- The 1:100 had ~20 colonies.
- The 1:1,000 had 7 colonies.

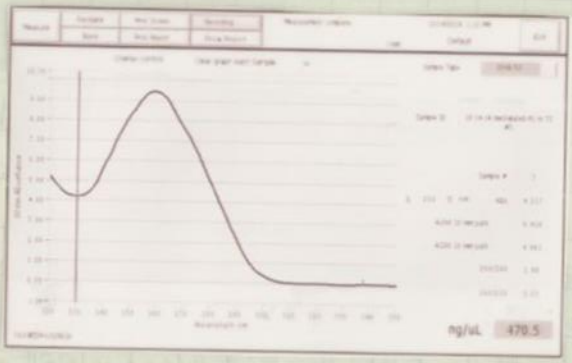
* Libraries were made *

10/14/14

DNA Extraction of PANI and P1

Using the Thermo Scientific Extraction kit, following DNA concentrations were obtained.





Digest of Methylated P1 in T3

500ng	P1
1	Eco R1
1	Pst 1
2	10 x Fast Digest Buffer
1	BSA
add to make 20 µL Total	PCR H_2O

* Use only one restriction enzyme (Eco R1) *

P1 #1, #2 + #3 – use 1 µL of DNA and 15 µL of H_2O (PCR).

- Place on heatbath for 45 min at 42°C.
- Place on heatblock for 15 min at 80°C.

Methylation Confirmation Gel

Row 1

Lane 1 – 1kb
Lane 2 – PAN 1
Lane 3 – P1
Lane 4 – P1 sample #1
Lane 5 – P1 Sample #2
Lane 6 – P1 Sample #3
Lane 7 – 1 kb
Lane 8 – Empty